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(54) Title: EFFECTIVE ANTAGONISTS OF THE LUTEINIZING HORMONE RELEASING HORMONE WHICH RELEASE NEGLIGIBLE HISTAMINE			
(57) Abstract			
<p>Antide is the decapeptide, N-Ac-D-S-Nal,D-pClPhe, D-3-Pal, Ser,NicLys, D-NicLys, Leu, Ilys, Pro, D-Ala,NH<sub>2</sub> which is an antagonist of luteinizing hormone releasing hormone (LHRH). This decapeptide, like others of the present in-vention, has high antioviulatory activity (AOA) and releases negligible histamine. Antide is scheduled for scale-up, safety testing and evaluation in the experimental primate and in clinical medicine. Numerous other peptides having structures re-lated to Antide were prepared and tested. These peptides had variations primarily in positions 5, 6, 7 and 8. Of these, N-Ac-D-2-Nal, D-pClPhe,D-3-Pal,Ser,PicLys,cis-DPzACAla,Leu,ILys,Pro,D-Ala-NH<sub>2</sub> was one of the most potent.</p>			

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EFFECTIVE ANTAGONISTS OF THE LUTEINIZING HORMONE RELEASING  
HORMONE WHICH RELEASE NEGLIBLE HISTAMINE

15

This is a continuation-in-part of U.S. Patent  
Application Number 088,431 filed August 24, 1987 which is  
incorporated by reference herein.

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was supported in part by the Contraceptive Branch of the  
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The present invention involves the design, synthesis  
and use of synthetic analogs of the luteinizing hormone  
releasing hormone (LHRH). An important achievement  
involved synthesis of analogs which functioned as  
30 antagonists of LHRH, were adequately potent to inhibit  
ovulation and allowed the release of only negligible  
amounts of histamine. Since there was no way of reliably  
forecasting the structure of an antagonist having high  
potency and very low histamine release, it was necessary  
35 to explore diverse approaches to discover a combination of  
structural features which would yield an antagonist of

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LHRH having high potency for ovulation inhibition and very low activity for histamine release.

Various peptides such as substance P, vasoactive  
5 intestinal peptide, gastrin, somatostatin, as well as  
others, are well known to cause the release of histamine  
from mast cells. These cells are in many tissues, such as  
skin, lung and mesentery, gingiva, etc. Most cells have  
granules containing histamine and other mediators of  
10 inflammation which can be released by peptides to cause  
capillary dilation and increased vascular permeability.  
When it was noted that an antagonist of LHRH, for example  
[Ac-D-2-Nal<sup>1</sup>, D-4-F-Phe<sup>2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>]-LHRH, caused edema  
of the face and extremities when it was administered to  
15 rats, it appeared likely that such antagonists, if  
administered to human subjects as a contraceptive agent,  
would cause serious edema of the face and elsewhere in the  
human body. Such side effects would likely prevent the  
administration of such antagonists to human subjects.

20

The histamine-containing leukocyte is a basophile  
which can also release histamine when stimulated by many  
of the same peptides mentioned above. Basophiles differ  
biochemically from mast cells and such differences may  
25 allow for both predictable and unpredictable histamine  
release in response to antagonists of LHRH. An antagonist  
of LHRH, to be used clinically to prevent ovulation,  
should not significantly release amounts of histamine from  
either mast cells or basophiles.

30

The discovery of the side effects such as the  
edematogenic and anaphylactoid actions of LHRH antagonists  
made desirable the discovery of new LHRH antagonists  
which prevented ovulation but did not release significant  
35 histamine. These undesirable side effects have been  
observed in rats, and it is likely that the Food and Drug

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Administration would not allow the testing of such antagonists in human subjects.

Karten et al. (4), have reviewed available knowledge  
5 on the structural characteristics for potent histamine  
release by antagonists of LHRH. Some of the most  
important findings are as follows. A most potent LHRH  
antagonist in triggering histamine release in vitro  
involved a combination of strongly basic D-amino acid side  
10 chains (Arg or Lys) at position 6 and in close proximity  
to Arg<sup>8</sup>, and a cluster of hydrophobic aromatic amino acids  
at the N-terminus. Thus, there is no specific amino acid  
of the ten amino acids which is solely responsible for  
histamine release. On the contrary, structural features  
15 ranging from the N-terminus (the amino acids in the first  
few positions, 1-4, etc.), and basic amino acids toward  
the C-terminus (positions 6 and 8) somehow participate in  
histamine release. Even D-Ala in position 10 has some  
influence on histamine release, the rationale for which is  
20 unclear. By themselves, two basic side chains in close  
proximity, as in positions 6 and 8, are insufficient alone  
to impart high release of histamine. The cluster of  
hydrophobic amino acids at the N-terminus is insufficient  
alone for high histamine releasing activity. Even a  
25 hexapeptide fragment has revealed moderate histamine  
releasing potency. There seems to be no correlation  
between antioovulatory potency and histamine release of  
these antagonists, in vitro.

30 In perspective, much of the entire chain of such  
decapeptide antagonists may have influence on histamine  
release. The same perspective appears to be true, but to  
different degrees, for high antioovulatory activity. These  
LHRH antagonists are usually decapeptides which indicates  
35 that there are ten variables to adjust for a desired  
anti-ovulatory activity and ten variables to adjust for

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eliminating histamine releasing activity. There are even further variations for each of these twenty variables, the number of possible peptides to design, synthesize and assay becoming incalculable. Presumably, some of the ten  
5 variables may be independent for anti-ovulatory activity and histamine releasing activity while some variables may overlap for these two biological activities. This situation poses extraordinary difficulties to solve before an antagonist of high potency for anti-ovulation and very  
10 low potency for histamine release could be produced.

Diverse structural changes and combinations of the ten amino acids followed by assays of both anti-ovulation and histamine release activities should be performed in  
15 the hope that a potent antagonist essentially free of side effects would be discovered. The synthesis of new amino acids to introduce into the decapeptide chains should also be explored since the commonly available amino acids might not suffice.

20

In the antagonists prepared according to the present invention, arginine and its derivatives were not utilized. Lysine was converted into derivatives with acyl groups or with alkyl groups on the E-amino group. The amino acid  
25 ornithine was acylated or alkylated on the d-amino group. Both the L- and D- forms of lysine and the L-form of ornithine were used in synthesizing these acyl and alkyl derivatives. Structurally related intermediates were also synthesized. All together, many new peptides were  
30 synthesized by the basic and minimal concepts of ten variables for anti-ovulation activity and ten variables for histamine release, which may be independent or partially overlapping. On such a basis, the number of such peptides that can be designed becomes overwhelming,  
35 and every reasonable priority must be considered to reduce

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the number of peptides to be synthesized in the hope that a discovery will be realized.

Certain peptides were synthesized, tested and found to demonstrate advantageous peptides. Among these desirable peptides were the following two.

[N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, ILys<sup>8</sup>, D-Ala<sup>10</sup>]-LHRH was effective to prevent ovulation and released remarkably little histamine.

[N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, PicLys<sup>5</sup>, D-PicLys<sup>6</sup>, ILys<sup>8</sup>, D-Ala<sup>10</sup>]-LHRH was twice as effective as the above peptide, and released no more histamine than do "super agonists" of LHRH, which are presently being marketed by several pharmaceutical companies.

These two new peptides, and yet additional related peptides described herein provide acceptable balances of high anti-ovulatory activity and low histamine release for full potential clinical utility.

The present invention involves the preparation and use of decapeptides having antioviulatory activity and with minimal histamine-releasing effects. These decapeptides includes those comprising:

Ser<sup>4</sup>, PicLys<sup>5</sup> and D-PicLys<sup>6</sup>;

N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, Ser<sup>4</sup>, D-PicLys<sup>5</sup> and Pro<sup>9</sup>;

N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, D-PicLys<sup>6</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>;

N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>;

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N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, Leu<sup>7</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>;

5 N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, Leu<sup>7</sup>, Pro<sup>9</sup> and D-Ser<sup>10</sup>;

D-pClPhe<sup>2</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>;

D-pClPhe<sup>2</sup>, Pro<sup>9</sup> and Ser<sup>10</sup>;

10

N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, ILys<sup>8</sup> and D-Ala<sup>10</sup>;

15 N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, ILys<sup>8</sup> and D-Ala<sup>10</sup>;

N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, PicLys<sup>5</sup>, D-PicLys<sup>6</sup>, ILys<sup>8</sup> and D-Ala<sup>10</sup>;

20 N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, IOrn<sup>8</sup> and D-Ala<sup>10</sup>;

N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, PicLys<sup>5</sup>, D-PicLys<sup>6</sup>, IOrn<sup>8</sup> and D-Ala<sup>10</sup>;

25

N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, MNicLys<sup>5</sup>, D-MNicLys<sup>6</sup>, IOrn<sup>8</sup> and D-Ala<sup>10</sup>;

30 N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, PzcLys<sup>5</sup>, D-PzcLys<sup>6</sup>, IOrn<sup>8</sup> and D-Ala<sup>10</sup>;

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N-Ac-D-pClPhe<sup>1</sup>, D-3-Pal<sup>3</sup>, Tyr<sup>5</sup>, D-NicLys<sup>6</sup> and ILys<sup>8</sup>;

N-Ac-D-Cl<sub>2</sub>Phe<sup>1</sup>, D-3-Pal<sup>3</sup>, Tyr<sup>5</sup>, D-NicLys<sup>6</sup> and ILys<sup>8</sup>;

5 acylated Lys<sup>5</sup>, D-acylated Lys<sup>6</sup> and N-alkylated diamino acid<sup>8</sup>;

NicLys<sup>5</sup>, D-NicLys<sup>6</sup> and ILys<sup>8</sup>;

10 PicLys<sup>5</sup>, D-PicLys<sup>6</sup> and ILys<sup>8</sup>;

NicLys<sup>5</sup>, D-NicLys<sup>6</sup> and IOrn<sup>8</sup>;

PicLys<sup>5</sup>, D-PicLys<sup>6</sup> and IOrn<sup>8</sup>;

15 MNicLys<sup>5</sup>, D-MNicLys<sup>6</sup> and IOrn<sup>8</sup>;

PzcLys<sup>5</sup>, D-PzcLys<sup>6</sup> and IOrn<sup>8</sup>;

20 Tyr<sup>5</sup>, D-NicLys<sup>6</sup> and ILys<sup>8</sup>;

Tyr<sup>5</sup>, D-NicLys<sup>6</sup> and IOrn<sup>8</sup>;

25 N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>; and

N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, PicLys<sup>5</sup>, cis D-PzACala<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.

30 The present invention further involves use of the above decapeptides in a process for inhibiting ovulation in an animal. This process comprises administering to said animal a decapeptide preferably having the structure: N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-  
35 NicLys<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>. Likewise, the inventive process may be used to inhibit ovulation in an

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animal; to inhibit the onset of puberty in an animal; to inhibit the sexual impetus of an animal; to alter the gonadal function of an animal; to inhibit the growth of hormone-dependent tumors in an animal; and to lower LH and  
5 FSH levels in serum of post-menopausal women. These and other related uses will be apparent to those skilled in the art upon examination of this specification.

Abbreviations and formulas used herein include the  
10 following:

	a	=	alpha
	BOC	=	t-butoxycarbonyl
	Br-Z	=	o-bromobenzyloxycarbonyl
15	nBuOAc	=	n-butylacetate
	n-BuOH	=	n-butanol
	c	=	<u>cis</u>
	CDCl <sub>3</sub>	=	deuteriochloroform
	CHCl <sub>3</sub>	=	chloroform
20	CH <sub>2</sub> Cl <sub>2</sub>	=	dichloromethane
	CH <sub>3</sub> CN	=	acetonitril
	Cl-Z	=	o-chlorobenzyloxycarbonyl
	d	=	delta
	DCC	=	dicyclohexylcarbodiimide
25	DIEA	=	diisopropylethylamine
	DMF	=	dimethylformamide
	E	=	eta
	Et	=	ethyl
	EtOAc	=	ethyl acetate
30	EtOH	=	ethanol
	Et <sub>2</sub> O	=	diethyl ether
	HF	=	hydrogen fluoride
	HOAc	=	acetic acid
	KH <sub>2</sub> PO <sub>4</sub>	=	potassium dihydrogen phosphate
35	MeOH	=	methanol
	MgSO <sub>4</sub>	=	magnesium sulfate

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	NH <sub>4</sub> OAc	=	ammonium acetate
	iPrOH	=	2-propanol
	py	=	pyridine
	t	=	<u>trans</u>
5	TFA	=	trifluoroacetic acid
	THF	=	tetrahydrofuran
	TOS	=	p-toluensulfonyl
	m	=	micro
	Z	=	benzyloxycarbonyl
10	Abu	=	2-aminobutyric acid
	Aile	=	alloisoleucine
	AnGlu	=	4-(4-methoxyphenylcarbamoyl)-2-aminobutyric acid
15	BzLys	=	N <sup>E</sup> -benzoyllysine
	Cit	=	citrulline
	Cl <sub>2</sub> Phe	=	3,4-dichlorophenylalanine
	CypLys	=	N <sup>E</sup> -cyclopentyllysine
	DMGLys	=	N <sup>E</sup> -N,N-dimethylglycyl)lysine
20	Dpo	=	N <sup>d</sup> -(4,6-dimethyl-2-pyrimidyl)ornithine
	Et <sub>2</sub> hArg	=	N <sup>G</sup> ,N <sup>G</sup> -diethylhomoarginine
	FPhe	=	4-fluorophenylalanine
	HOBLys	=	N <sup>E</sup> -(4-hydroxybenzoyl)lysine
25	Ilys	=	N <sup>E</sup> -isopropyllysine
	INicLys	=	N <sup>E</sup> -isonicotinoyllysine
	IOrn	=	N <sup>d</sup> -isopropylornithine
	Me <sub>3</sub> Arg	=	N <sup>G</sup> ,N <sup>G</sup> ,N <sup>G1</sup> -trimethylarginine
	Me <sub>2</sub> Lys	=	N <sup>E</sup> ,N <sup>E</sup> -dimethyllysine
30	MNicLys	=	N <sup>E</sup> -(6-methylnicotinoyl)lysine
	MPicLys	=	N <sup>E</sup> -(6-methylpicolinoyl)lysine
	NACala	=	3-(4-nicotinoylaminocyclohexyl)alanine
	2-Nal	=	3-(2-naphthyl)alanine
	NicLys	=	N <sup>E</sup> -nicotinoyllysine
35	NicOrn	=	N <sup>d</sup> -nicotinoylornithine
	Nle	=	norleucine, 2-aminohexanoic acid

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	NMeLeu	=	N-methyllleucine
	Nval	=	norvaline, 2-aminopentanoic acid
	3-Pal	=	3-(3-pyridyl)alanine
	pClPhe	=	3-(4-chloro)phenylalanine
5	PicLys	=	N <sup>E</sup> -picoloyllysine
	Pip	=	piperidine-2-carboxylic acid
	PmcLys	=	N <sup>E</sup> -(4-pyrimidinylcarbonyl)lysine
	PmACAla	=	3[4(4-pyrimidinylcarbonyl)aminocyclohexyl]alanine
10	PzACAla	=	3(4-pyrazinylcarbonylaminocyclohexyl)alanine
	3-PzAla	=	3-pyrazinylalanine
	PzcLys	=	N <sup>E</sup> -pyrazinylcarbonyllysine
15	Sar	=	N-methylglycine
	TinGly	=	3-thienylglycine

Most natural amino acids were obtained from Peninsula Laboratories, San Carlos, CA. The hydroxyl group of Ser was protected as the benzyl ether, the phenolic hydroxyl group of Tyr as the Br-Z derivative, and E-amino group of Lys as the Cl-Z derivative, the guanidino group of Arg and the imidazole group of His as the TOS derivatives. The α-amino function was protected as the BOC derivative.

25 BOC-Orn(Z) was obtained from Sigma Chemical Co., St. Louis, Mo. BOC-D-2-Nal, BOC-D-3-Pal, BOC-D-Cl<sub>2</sub>Phe, BOC-pClPhe and BOC-ILys(Z) dicyclohexylamine salt were provided by the Southwest Foundation for Biomedical Research, San Antonio, TX. The benzhydrylamine

30 hydrochloride resin was obtained from Beckman Bioproducts, Palo Alto, CA. The nitrogen content was about 0.65 mmoles/g. The CH<sub>2</sub>Cl<sub>2</sub> was distilled before use.

The present invention involves the design, synthesis and use of LHRH antagonists with high antioviulatory potency and diminished activity to release histamine (1).

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These new antagonists feature, for example, D-N<sup>E</sup>-nicotinoyllsine (D-NicLys) in position 6 and N<sup>E</sup>-isopropyllysine (ILys) in position 8. The solution of D-Arg<sup>6</sup>, particularly in combination with Arg<sup>8</sup> and a cluster of hydrophobic aromatic amino acid residues at the N-terminal, have been implicated in the release of histamine (2-4).

Other reductions of anaphylactoid activity were obtained by increasing the distance between the positive charges in positions 6 and 8 by Arg<sup>5</sup> and by inclusion of a neutral residue in position 6 as in [N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Arg<sup>5</sup>, D-4(p-methoxybenzoyl)-2-amino-butyric acid<sup>6</sup>, D-Ala<sup>10</sup>]-LHRH (2-Nal represents 3-(2-naphthyl) alanine; pClPhe represents 3-(4-chlorophenyl)alanine; 3-Pal represents 3-(3-pyridyl)alanine) by Rivier et al. (5) and [N-Ac-D-2-Nal<sup>1</sup>, D-aMepClPhe<sup>2</sup>, D-Trp<sup>3</sup>, Arg<sup>5</sup>, D-Tyr<sup>6</sup>, D-Ala<sup>10</sup>]-LHRH (aMepClPhe represents 2 methyl-3-(4-chlorophenyl)alanine) by Roeske et al. (6). Further modifications in position 6 are reductive alkylation of D-Lys<sup>6</sup> by Hocart et al. (7), incorporation of N,N-diethylhomoarginine by Nestor et al. (9). The cyclic analogs recently synthesized by Rivier et al. did not show any lowering in histamine release compared to the linear counterparts (10).

From the peptides of the present invention, two were initially selected as models for further design. The peptide [N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, ILys<sup>8</sup>, D-Ala<sup>10</sup>]-LHRH (named Antide) had an impressive combination of potency and low histamine release; antioviulatory activity (AOA) was 100% at 1ug and 36% at 0.5ug; ED<sub>50</sub> for histamine release, in vitro, was consistently above 300ug/ul as compared to about 0.17 for the standard analog [N-Ac-D-2-Nal<sup>1</sup>, D-pFPhe<sup>2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>]-LHRH (pFPhe represents 3-(4-fluorophenyl)alanine)

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(5). Another analog was identical to Antide except for PicLys<sup>5</sup> and D-PicLys<sup>6</sup> (PicLys represents N-picoloyllysine); 100% AOA at 0.5ug and 40% at 0.25ug; ED<sub>50</sub>, 93±11.

5

Included herein are results from LHRH analogs with acylated aminocyclohexylalanine residues in position 6, from analogs in which Leu<sup>7</sup> has been substituted with other neutral residues, from a comparison of ILys<sup>8</sup> vs. IOrn<sup>8</sup>, and from tests on oral activity and duration of antagonists activity when administered orally or parenterally (s.c.)

Melting points are uncorrected. NMR data are reported as  $\delta$ -values downfield from TMS.

15

Before acylation, the Z and Cl-Z groups of Lys and Orn were cleaved by hydrogenolysis in MeOH in the presence of 10% Pd/C.

20

BOC-D-BzLys was synthesized by acylation of BOC-D-Lys with benzoyl chloride as described for the L- isomer by Bernardi *et al.* (17).

25

BOC-DMG-Lys was prepared by acylation of BOC-Lys with chloroacetyl chloride using the same method and the reacting the crude product from 10 mmoles BOC-Lys in 10 ul THF with 10 ul 40% aq. dimethylamine. The reaction mixture was stirred 15 minutes in ice bath and then 2.5 hours at room temperature. After evaporation in vacuo the crude product was dissolved in 10 ul H<sub>2</sub>O and applied on a Bio-Rad AG1-X8 column, acetate form, 1 x 25 cm. The column was first washed with 200 ul water and then the product was eluted with 6% HOAc and lyophilized several times to remove the HOAc. Yield 60-70%. Amorphous mass. R<sub>f</sub> (n-BuOH:py:HOAc:H<sub>2</sub>O = 30:10:3:12) = 0.27. Purity >

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95%. NMR ( $\text{CDCl}_3$ ): 1.45, s, 9H, t-butoxy group; 1.85-1.48, m, 6H, B, y, d,  $\text{CH}_2$  groups; 2.6, s, 6H,  $\text{N}(\text{CH}_3)_2$ ; 3.25, m, 2H, E- $\text{CH}_2$ ; 3.37, s, 2H, N- $\text{CH}_2$ -CO; 4.15, m, 1H, a-CH.

5 The other acylated Lys derivatives in the tables were prepared from BOC-D or L-Lys and the corresponding p-nitrophenyl ester.

p-Nitrophenyl nicotinate. To 9.85 g, 80 mmoles, 10 nicotinic acid and 13.35 g, 96 mmoles p-nitrophenol in 250 ul DMF was added 16.5 g, 80 mmoles DCC with stirring in ice-bath. After 1 hour at 0°C and 3 hours at room temperature the urea was filtered off and the product was precipitated by the addition of an equal volume of water. 15 Filtration, drying in vacuo and recrystallization from i-PrOH gave 11.22 g, 57% of white needles, m.p. 172.5-173°C (24)

p-nitrophenyl isonicotinate was prepared, in the same 20 manner 12 g, 61%, m.p. 139-141°C, m.p. 137-139°C. (18)

Also p-nitrophenyl 6-methylnicotinate was prepared in the same way. Yield from 70 mmoles 6-methylnicotinic acid: 6.0 g, 33% after recrystallization from MeOH. M.p. 25 156-157°C.  $R_f$  (2% MeOH in  $\text{CHCl}_3$ ) = 0.57 NMR ( $\text{CDCl}_3$ ): 2.7, s, 3H,  $\text{CH}_3$ ; 7.36, d, 1H, py  $\text{H}^5$ ; 7.45, m, 2H, H adjacent to the oxygen in the phenyl ring; 8.34, m, 3H, H adjacent to the  $\text{NO}_2$  group in the phenyl ring overlapping with py  $\text{H}^4$ ; 9.27, d, 1H, py  $\text{H}^2$ .

30 p-nitrophenyl picolinate. 4.92 g, 40 mmoles, picolinic acid and 5.84 g, 42 mmoles p-nitrophenol were suspended/dissolved in 200 ul  $\text{CH}_2\text{Cl}_2$ . Then 8.24 g 40 mmoles, DCC was added in 20 ul  $\text{CH}_2\text{Cl}_2$  with vigorous 35 stirring. Stirring was continued in room temperature for 17 hours. Then the mixture was filtered and the filter

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cake washed with 30-40 ul  $\text{CH}_2\text{Cl}_2$ . The raw product was first treated with 100 ul  $\text{Et}_2\text{O}$  with stirring in ice-bath and filtered. Recrystallization from 250 ul  $\text{iPrOH}$  gave 6.24 g, 63% product. M.p.  $154-6^\circ\text{C}$  (dec.). M.p.  $145-7^\circ\text{C}$  (18).

Pyrazinecarboxylic acid p-nitrophenylester. This compound was prepared using the same method as the previous compound. From 40 mmoles pyrazinecarboxylic acid and 44 mmoles p-nitrophenol was obtained 35.2 mmoles, 88%, ester. M.p.  $180-182^\circ\text{C}$  (dec.).  $R_f$  ( $\text{CHCl}_3:\text{MeOH} = 49:1$ ) = 0.72. NMR ( $\text{CDCl}_3$ ): 7.5,m and 8.37m, 2H each, hydrogens adjacent to the oxygen and nitro group respectively in the phenol ring; 8.84,m, 1H, pyrazine  $\text{H}^5$ ; 8.9,d, 1H, pyrazine  $\text{H}^6$ ; 9.48,d, 1H, pyrazine  $\text{H}^3$ .

BOC-NicLys. 2.5 g BOC-Lys (L or D) was suspended in 200 ul DMF with stirring. Then 1.1 equivalent of p-nitrophenyl nicotinate was added and the mixture stirred at room temperature for 36 hours. The mixture was then filtered and the filtrate evaporated to dryness at reduced pressure to yield a yellow oil. The residue was stirred with 2x50 ul  $\text{Et}_2\text{O}$  in ice-bath. The first  $\text{Et}_2\text{O}$  phase was decanted, the second was filtered off. Recrystallization from EtOAc/hexanes gave 2.05 g product, 58% (L-form). M.p.  $138^\circ\text{C}$ , lit. (17)  $138-141^\circ\text{C}$ . L-form  $[\alpha]_D^{20} = -2.91^\circ$  (MeOH), D-form  $[\alpha]_D^{20} = 3.35^\circ$  (MeOH).

L- and D-BOC-INicLys were prepared similarly by acylating 10 mmoles L or D BOC-Lys with p-nitrophenyl isonicotinate in 100 ul DMF, 40 hours, room temperature. The crude product was partitioned between 120 ul EtOAc and 50 ul  $\text{H}_2\text{O}$ . The EtOAc phase was extracted with 2 x 50 ul  $\text{H}_2\text{O}$  and 50 ul brine. The original aqueous phase was back-extracted with 30 ul EtOAc. The combined EtOAc phases were then dried ( $\text{MgSO}_4$ ) and evaporated and the

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residue was treated with Et<sub>2</sub>O and recrystallized as above to give 1.07 g, BOC- L-INicLys, 30.5%. The yield for the D compound was 1.26 g, 36%. NMR (Acetone d<sub>6</sub>):

- 1.4, s, 9H, t-butoxy group; 1.8-1.48, m, 6H, B, y, d, -CH<sub>2</sub>-;  
5 3.44, t, 2H, E-CH<sub>2</sub>; 4.13, m, 1H, a-CH; 7.77, m, 2H, py H<sup>5</sup> and H<sup>3</sup>;  
8.70, m, 2H, py H<sup>2</sup> and H<sup>6</sup>.

L- and D-BOC-PicLys. 1.23 g, 5 mmoles, of L- or D-BOC-Lys was stirred with 1.34 g, 5.5 mmoles, p-nitrophenyl  
10 picolinate in 60 ul DMF for 16 hours. After filtration and evaporation and product was purified by column chromatography on silica gel on a 4.5 x 32 cm column and the solvent system n-BuOH:py:HOAc:H<sub>2</sub>O = 30:10:3:12. The product after chromatography was dissolved in EtOAc and  
15 washed with H<sub>2</sub>O, brine, dried and evaporated in vacuo. The yields were usually 60-70%. NMR (CDCl<sub>3</sub>):

- 1.43, s, 9H, t-butoxy group; 1.73-1.45, m, 6H, B, y, d-CH<sub>2</sub>;  
3.47, m, 2H, E-CH<sub>2</sub>; 4.32, m, 1H, a-CH; 7.43, m, 1H, py H<sup>5</sup>;  
7.85, m, 1H, py H<sup>4</sup>; 8.2, m, 1H, py H<sup>3</sup>; 8.55, m, 1H, py H<sup>6</sup>.

20

L- and D-BOC-MNicLys. 10 mmoles BOC-Lys and 10.5 mmoles p-nitrophenyl 6-methylnicotinate were allowed to react in 150 ul DMF in the usual manner. After 27 hours filtration and evaporation yielded a yellow oil. Et<sub>2</sub>O  
25 treatment (2 x 50 ul) gave 3.3 g product which was recrystallized from 50 ul 20% MeOH in EtOAc/hexane. Yield 2.87 g, 78.6% (L-form). R<sub>F</sub>(n-BuOH:py:HOAc:H<sub>2</sub>O = 32:10:3:12) = 0.61. NMR(CDCl<sub>3</sub>): 1.46, s, 9H, t-butoxy group; 1.9-1.5, m, 6H, B, y, d-CH<sub>2</sub>; 2.57, s, 3H, py CH<sub>3</sub>; 3.36, m, 2H, E-CH<sub>2</sub>;  
30 4.11, m, 1H, a-CH; 7.22, d, 1H, py H<sup>5</sup>; 8.08, m, 1H, py H<sup>4</sup>;  
8.95, broad s, 1H, py H<sup>2</sup>.

L- and D-BOC-PzcLys. Using the method above was obtained from 7.7 mmoles pyrazine carboxylic acid p-nitrophenyl ester and 7 mmoles BOC-Lys, L or D, in 100 ul  
35 DMF about 6 mmoles product after recrystallization from

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iPrOH.  $R_f(n\text{-BuOH:py:HOAc:H}_2\text{O} = 30:10:3:12) = 0.47$ . NMR ( $\text{CDCl}_3$ ): 1.45, s, 9H, t-butoxy group; 1.9-1.48, m, 6H, B, y, d- $\text{CH}_2$ -; 3.51, m, 2H, E- $\text{CH}_2$ ; 4.29, m, 1H, a-CH; 8.52, q, 1H, pyrazine  $\text{H}^5$ ; 8.77, d, 1H, pyrazine  $\text{H}^6$ ; 9.41, d, 1H, pyrazine  $\text{H}^3$ .

5

BOC-L-NicOrn. This compound was prepared the usual way by reacting 7 mmols p-nitrophenyl nicotinate with 5 mmols BOC-Orn in 75  $\mu\text{l}$  DMF for 36 hours. Evaporation and recrystallization from EtOAc gave 3.5 mmols, 70%, NicOrn, m.p. 143-144°C.  $R_f(n\text{-BuOH:HOAc:H}_2\text{O} = 4:1:2) = 0.70$ . NMR( $\text{CDCl}_3$ ): 1.45, s, 9H, t-butoxy group; 7.46, m, 1H, py  $\text{H}^5$ ; 8.27, m, 1H, py  $\text{H}^4$ ; 8.69, m, 1H, py  $\text{H}^6$ ; 9.05, m, 1H, py  $\text{H}^2$ .

BOC-D-trans-NACAla. 1.43 g, 5 mmols, BOC-D-trans-3(4-aminocyclohexyl) alanine (provided by the Southwest Foundation for Biomedical Research) was stirred with 1.35 g, 5.5 mmols, p-nitrophenyl nicotinate in 60  $\mu\text{l}$  DMF for 120 hours in room temperature. The mixture was then filtered, evaporated, treated with  $\text{Et}_2\text{O}$  in ice bath and filtered again. Recrystallization was done by heating in 12  $\mu\text{l}$  EtOH and adding 18  $\mu\text{l}$  hot  $\text{H}_2\text{O}$ . This produced a clear solution from which crystals separated on cooling. This procedure was repeated twice. Yield: 0.98 g, 50%. Purity >95%. M.p. >220°C. NMR( $\text{DMSO } d_6$ ): 1.46, s, 9H, t-butoxy group; 1.9-1.48, m, 11H, ring  $\text{CH}_2$ , ring CH in position 1 and B- $\text{CH}_2$ ; 3.72, m, 1H, ring CH in position 4; 3.95, m, 1H, a-CH; 7.48, m, 1H, py  $\text{H}^5$ ; 8.16, m, 1H, py  $\text{H}^4$ ; 8.67, m, 1H, py  $\text{H}^6$ ; 8.96, m, 1H, py  $\text{H}^2$ .

BOC-D-cis-NACAla. 5 mmols BOC-D-cis-3(4-aminocyclohexyl)alanine (source: as above) and 5.5 mmols p-nitrophenyl nicotinate were allowed to react in DMF as above. Reaction time: 25 hours. Purification was achieved by  $\text{Et}_2\text{O}$  treatment as above and silica gel chromatography on a 4.5 x 32 cm column using the solvent system  $\text{CHCl}_3:\text{MeOH:py:HOAc} = 75:10:10:5$ . Yield 1.3 g, 61%.

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amorphous powder.  $R_f$  (column system) = 0.58. NMR ( $CDCl_3$ ): 1.44, s, 9H, t-butoxy group; 1.95-1.45, m, 11H, ring  $CH_2$ , ring CH in position 1 and B- $CH_2$ ; 4.22, m, 1H, a-CH; 4.35, m, 1H, ring CH in position 4: 7.35, 8.24, 8.63 and 8.98, 1H each, assignments as previous compound.

BOC-IOrn(Z). This compound was prepared from BOC-Orn(Z) by reductive alkylation with acetone and  $H_2$ /Pd as described by Prasad *et al.* (23) followed by conversion to the Nd- Z derivative with benzyl chloroformate in aqueous alkali (Schotten-Baumann conditions). Purification was achieved by chromatography on silica gel with  $CHCl_3$ /MeOH 85:15.  $R_f$  ( $CHCl_3$ :MeOH:HOAc = 85:15:3) = 0.8. NMR( $CHCl_3$ ): 1.10, d, 6H, isopropyl  $CH_3$ ; 1.40, s, 9H, t-butoxy group; 1.7-1.5, m, 4H, B,  $\gamma$ - $CH_2$ ; 3.09, m, 2H, d- $CH_2$ ; 4.2, m, 1H, a-CH; 5.10, s, 2H, benzyl  $CH_2$ ; 7.3, m, 5H, aromatics.

BOC-CypLys(Z). 2.04 g BOC-Lys(Z) was dissolved in 8 ul of cyclopentanone and 32 ul  $H_2O$  containing 0.22 g NaOH. Hydrogenation was performed in the presence of 0.4 g 10% Pd/C at 50-60 psi in a Parr apparatus. After 4 hours the hydrogenation was interrupted and 2 ul 0.5 M NaOH and 10 ul MeOH were added. The hydrogenation was then continued for 16 hours at 50-60 psi. Then filtration and evaporation. The residue was dissolved in 75 ul  $H_2O$  and the aqueous phase extracted with three times with  $Et_2O$  and once with hexane. The pH was then brought to 6-7 with HCl and the solution evaporated in rotary evaporator, bath temperature 40°C. The resulting product was then converted to the Z-derivative using benzyl chloroformate in aqueous NaOH (Schotten-Baumann conditions). Yield: 1.3 g, 58% overall.  $R_f$  (n-BuOH:py:HOAc: $H_2O$  - 30:10:3:12) = 0.69. Purity >95%. NMR ( $CDCl_3$ ): 1.45, s, 9H, t-butoxy group; 1.95-1.35, m, 14H, ring  $CH_2$  + B,  $\gamma$ , d- $CH_2$ ; 3.13, broad t, 2H, E- $CH_2$ ; 4.34-4.05, m, 2H, a-CH + ring CH; 5.13, s, 2H, benzyl  $CH_2$ ; 7.35, m, 5H, aromatic protons.

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BOC-Me<sub>2</sub>Lys, D- and L-. These compounds were prepared by hydrogenolysis of the corresponding Z- or Cl-Z- derivatives in the presence of 37% formaldehyde essentially as described by L. Benoiton (22) for the N<sup>a</sup> - acetyl analog. Purification was achieved by chromatography on silica gel with the solvent system n-BuOH:py:H<sub>2</sub>O = 2:2:1. The yields are 40-65% and the products are amorphous. NMR (CDCl<sub>3</sub>): 1.41, s, 9H, t-butoxy group; 1.9-1.5, m, 6H, B, γ, δ-CH<sub>2</sub>; 2.6, s, 6H, N(CH<sub>3</sub>)<sub>2</sub>; 2.8, m, 2H, E-CH<sub>2</sub>; 4.03, m, 1H, α-CH.

BOC-D-AnGlu. 0.62 g, 3 mmoles, DCC was added to the ice-cooled solution of 1.10 g, 3 mmoles, BOC-D-glutamic acid α-benzylester and 0.39 g, 3 mmoles, p-anisidine in 25 ul CH<sub>2</sub>Cl<sub>2</sub>. The reaction mixture was stirred while warming up to room temperature and then another 17 hours. The dicyclohexylurea was then filtered off and CHCl<sub>3</sub> added to a total volume of 125 ul. This solution was extracted with 2 x 1N H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O, saturated NaHCO<sub>3</sub>, 2 x H<sub>2</sub>O and dried (MgSO<sub>4</sub>). Evaporation and recrystallization from EtOH gave 0.99 g, 74% product, m.p. 129.5-131°C. R<sub>f</sub> (4% MeOH in CHCl<sub>3</sub>) = 0.53. This product was dissolved in 30 ul MeOH and 10 ul EtOH and hydrogenated in the presence of 0.3 g Pd/C at 50 psi for 2.5 hours. Filtration and evaporation gave a quantitative yield of BOC-D-AnGlu. Not crystalline. Purity >98%. NMR (CDCl<sub>3</sub>): 1.45, s, 9H, t-butoxy group; 2.35-1.95, m, 2H, B-CH<sub>2</sub>; 2.6-2.4, m, 2H, γ-CH<sub>2</sub>; 3.76, s, 3H, OCH<sub>3</sub>; 4.3, m, 1H, α-CH; 6.82 and 7.42, broad d, 2H each, aromatic protons.

30

BOC-Me<sub>3</sub>Arg. First, N,N,N',S-tetramethylisothiourea was prepared by the procedure of Lecher and Hardy (19). B.p. (15 mm) = 74°C, lit(above) 68°C at 11 mm. BOC-Orn, 9 mmoles, and tetramethylisothiourea, 10 mmoles, were dissolved in 15 ul DMF and 2 ul triethylamine and incubated at 100°C for 2 hours and at room temperature for

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10 hours. Then the reaction mixture was evaporated to dryness and passed through a silica gel column eluted by iPrOH:triethylamine:H<sub>2</sub>O = 42:6:13. The white solid so obtained was dissolved in H<sub>2</sub>O and the solution was

- 5 acidified with 6N HCl and lyophilized to give 5.5 mmoles product.  $R_f$  (column eluant) = 0.50. NMR (D<sub>2</sub>O): 1.42, s, 9H, t-butoxy group, 2.80, m, 1H,  $\alpha$ -CH; 2.89, s, 3H, CH<sub>3</sub> on guanidino group; 2.96, s, 6H, (CH<sub>3</sub>)<sub>2</sub>N; 3.25, t, 2H,  $\delta$ -CH<sub>2</sub>; 1.50, m, 4H,  $\beta$ ,  $\gamma$ -CH<sub>2</sub>.

10

BOC-Dpo. From 10 mmoles arginine hydrochloride and 1.72 g sodium hydrogen carbonate dissolved in 17 ul H<sub>2</sub>O, 28.6 ul acetylacetone and 28.6 ul EtOH was obtained 7.5 mmoles Dpo following the procedure of F.-S. (20). The

- 15 product was then converted to the corresponding BOC-derivative using di-t-butyl dicarbonate in 50% aqueous dioxane in the presence of sodium hydroxide. This reaction proceeds in essentially quantitative yield.  $R_f$  (nBuOH:HOAc:H<sub>2</sub>O = 4:1:2) = 0.63. NMR (CDCl<sub>3</sub>): 20 1.45, s, 9H, t-butoxy group; 1.9-1.5, 4H,  $\beta$ ,  $\gamma$ -CH<sub>2</sub>; 2.33, s, 6H, CH<sub>3</sub>; 3.46, m, 2H,  $\delta$ -CH<sub>2</sub>; 4.24, m, 1H,  $\alpha$ -CH; 6.35, s, 1H, aromatic H. L- and D- forms react similarly.

- BOC-D-Et<sub>2</sub>hArg. This compound was prepared by the 25 method of Nestor and Vickery, U.S. Pat. 4,530,920, July 23, 1985.  $R_f$  (nBuOH:HOAc:H<sub>2</sub>O = 4:1:2) = 0.52.

- The peptides of the present invention were synthesized by the solid phase method using a Beckman 30 Model 990 Peptide Synthesizer. (1, 11) The benzhydrylamine hydrochloride resin (BHA-resin) was used as a solid support. The program of the synthesizer was divided into subprograms.

- 35 1. Deprotection: 1. CH<sub>2</sub>Cl<sub>2</sub> (2 x wash, 1 or 2 min); 2. 50% TFA in CH<sub>2</sub>Cl<sub>2</sub> containing 0.1% indole (1 x

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wash, 1 or 2 min); 3. 50% TFA in  $\text{CH}_2\text{Cl}_2$  containing 0.1% indole (deprotection, 20 min); 4.  $\text{CH}_2\text{Cl}_2$  (2 x wash).

2. Neutralization: 1.  $\text{CH}_2\text{Cl}_2$  (2 x wash, 1 or 2 min); 2. DIEA (10% in  $\text{CH}_2\text{Cl}_2$ ) (2 x wash, 1 or 2 min); 3. DIEA (10% in  $\text{CH}_2\text{Cl}_2$ ) (neutralization, 5 min); 4.  $\text{CH}_2\text{Cl}_2$  (2 x wash, 1 or 2 min).

3. DCC Coupling: 1.  $\text{CH}_2\text{Cl}_2$  (2 x wash, 1 or 2 min); 2. amino acid solution in  $\text{CH}_2\text{Cl}_2$  (delivery, transfer, mix, 5 min); 3. DCC (10% in  $\text{CH}_2\text{Cl}_2$ , (delivery and mix, 180 min); 4.  $\text{CH}_2\text{Cl}_2$  (2 x wash, 1 or 2 min).

4. Active Ester Coupling: not used.

15

5. Final Wash: 1.  $\text{CH}_2\text{Cl}_2$  (2 x wash, 1 or 2 min); 2. i-PrOH (3 x wash, 1 or 2 min); 3. DMF (3 x wash, 1 or 2 min); 4.  $\text{CH}_2\text{Cl}_2$  (3 x wash, 1 or 2 min).

20 6. Wash after TFA Treatment: 1.  $\text{CH}_2\text{Cl}_2$  (2 x wash, 1 or 2 min); 2. i-PrOH (2 x wash, 1 or 2 min);  $\text{CH}_2\text{Cl}_2$  (3 x wash, 1 or 2 min).

25 7. Acetylation: 1.  $\text{CH}_2\text{Cl}_2$  (2 x wash, 1 or 2 min); 2. 25%  $\text{Ac}_2\text{O}$  and Py in  $\text{CH}_2\text{Cl}_2$  (1 x wash, 1 or 2 min); 3. 25%  $\text{Ac}_2\text{O}$  and Py in  $\text{CH}_2\text{Cl}_2$  (acetylation, 20 min); 4.  $\text{CH}_2\text{Cl}_2$  (2 x wash, 1 or 2 min).

30 The first amino acid was attached to the resin by the program sequence 2-3-5. Before placing the resin into the reaction vessel, the resin was washed in a separatory funnel with 25  $\mu\text{l}$   $\text{CH}_2\text{Cl}_2$ /g resin to remove the fine particles. In all couplings, usually a 3-4 fold excess of the Boc-amino acid over the nitrogen content of the resin  
35 was used. This procedure generally resulted in a complete coupling reaction. If a positive ninhydrin color reaction

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was observed, a second coupling was performed (program sequence 3-5). Then, the resin was acetylated (program sequence 7-5).

5        The next amino acid was attached by the program sequence 1-6-2-3-5. For DCC coupling, all amino acids were dissolved in  $\text{CH}_2\text{Cl}_2$ . Acetylation of the amino acid residue in position 1 was performed using the program sequence 1-6-2-7-5. The volume of the solvents and the  
10       reagents used for the washing and the performing of the chemical reactions was about 10 ul/g resin.

After all of the amino acids had been coupled, the peptide resin was dried overnight, in vacuo. The resin  
15       was then treated with double-distilled liquid hydrogen fluoride (10 ul/g resin) containing 10-25% distilled anisole or p-cresol for 1 hour at 0°C. Then, the HF was evaporated under reduced pressure and the residue was dried overnight, in vacuo, by an oil pump. The mixture  
20       was then extracted several times with  $\text{Et}_2\text{O}$  (25 ul/g resin), then with aqueous. HOAc, 30%, 50%, 10%, and once with 25 ul distilled, deionized water. The combined aqueous solution was lyophilized to yield the crude peptide.

25

Most peptides were purified by silica gel chromatography (1 x 60 cm column) using one of the solvent systems  $\text{nBuOH}:\text{HOAc}:\text{H}_2\text{O} = 4:1:2$  or  $4:1:5$  upper phase or  $\text{nBuOAc}:\text{nBuOH}:\text{HOAc}:\text{H}_2\text{O} = 2:8:2:3$  followed by gel filtration  
30       over Sephadex G 25 with 6% HOAc as the eluant. In the case of unsatisfactory purity after this procedure the peptides were further purified by semipreparative HPLC using a Waters liquid chromatograph equipped with a 660 solvent programmer. A 1.2 x 25 cm m-Bondapak  $\text{C}_{18}$  column  
35       was used with the solvent system A = 0.1 M  $\text{NH}_4\text{OAc}$  pH 5.0 and B = 20% A + 80%  $\text{CH}_3\text{CN}$ . Different gradients of

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increasing amounts of B in 15 - 25 minutes were employed to effect purification.

An alternate purification scheme has been gel  
5 filtration over Sephadex G-25 with 6% HOAc followed by chromatography over Sephadex LH 20 (2.5 x 100 cm) with the solvent system  $H_2O:nBuOH:HOAc:MeOH = 90:10:10:8$ . If necessary, the latter procedure was repeated 1 - 2 times.

10 The purity of the peptides was assessed by thin layer chromatography on Merck silica gel plates in at least four different solvent systems as shown in Table II. The spots were developed with the chlorine/o-tolidine reagent. In Table II are also shown the conditions and results of  
15 analytical HPLC. The equipment was the one described above except that an analytical  $\mu$ -Bondapak  $C_{18}$  column (3.9 mm x 30 cm) was used.

Amino acid analyses were performed on a Beckman model  
20 118 CL amino acid analyzer. Samples of about 0.5 ug were hydrolyzed in 6N hydrochloric acid in sealed glass tubes for 24 hours at 110°C. The residue was then evaporated and dissolved in citrate buffer, pH 2.2 and applied to the analyzer. The results are in Table III.

25 The antioviulatory activity, AOA, in rats was determined as described by Humphries et al. (12). The wheal test was performed by intradermally injecting 10 ug of peptide in 100 ul of saline into anaesthetized rats,  
30 measuring the ideally circular wheal response and calculating the area. The in vitro histamine release test was done as described by Karten et al. (4).

The results of these bioassays are presented in Table  
35 I and other Tables appended hereto.

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Of the 57 peptides in Table I, 21 had an AOA of about 90% or more at a dosage of 1 ug in the present assay. Of the 37 peptides of Table 1 tested for histamine release in the rat mast cell assay, 10 had  $E_{D50}$  values of 300 or more as compared to 0.17 for the standard compound [N-Ac-D-2-Nal<sup>1</sup>, D-4-F-Phe<sup>2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>]-LHRH. Nine additional analogs had  $E_{D50}$  values ranging from 86 to 288, i.e. they do not release more histamine than clinically used "superagonists".

10

Of the thirty-seven peptides of Table 1 tested in the rat mast cell assay, seven (numbers 4, 23, 24, 43 (Antide), 44, 53, 55) had both an AOA of about 90% or more at 1 ug and an  $E_{D50}$  value of about  $\geq 86$  ug/ul. This included the potent analog, No. 53, which had 100% AOA at 0.5 ug and 40% AOA at 0.25 ug. The  $E_{D50}$  value for this analog was  $93 \pm 28$ . It was thus demonstrated that high AOA with low histamine release could be found in the analogs of the present invention.

20

Structural features in common for these seven peptides are: 1) A D-Lys residue in position 6 which was acylated by the weakly basic nicotinic acid or analogs like picolinic and 6-methylnicotinic acid. 2) The corresponding acylated L-Lys residue or the natural Tyr in position 5. 3) The alkylated derivatives ILys or IOrn in position 8. 4) Arg is absent from the sequence.

Two examples of the influence of Arg on histamine release are the pairs 43, 10 and 4, 1. No. 43 (Antide) has the sequence N-Ac-D-2-Na<sup>1</sup>, D-pClPhe<sup>2</sup><sub>sub</sub>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup>, D-Ala<sup>10</sup>-NH<sub>2</sub>. Its  $E_{D50}$  value is  $>300$ . No. 10 is identical in sequence except that NicLys<sup>5</sup> is replaced by Arg<sup>5</sup>. This caused the  $E_{D50}$  value to decrease to  $4.3 \pm 0.52$ . No. 4 has identical sequence as No. 43 except for Tyr in position 5. Its  $E_{D50}$

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value is  $133 \pm 22$ . In No. 1, ILys<sup>8</sup> in this sequence is replaced by Arg<sup>8</sup> which caused the E<sub>D50</sub> value to decrease to  $39.2 \pm 7$ . It thus seems that position 5 is more sensitive than position 8 for Arg substitution.

5

In position 8, the alkylated ILys and IOrn residues are superior to Lys and Orn, respectively, both with respect to AOA and histamine release (pairs 3,4 and 6,7). Whether ILys<sup>8</sup> or IOrn<sup>8</sup> is best seems to be sequence

10 dependent.

For the determination of duration of action, the antagonist was administered s.c. or orally to 26 days old female rats at a specific time before administration of the agonist, [D-Qal<sup>6</sup>]-LHRH. The serum levels of rat luteinizing hormone (LH) and rat follicle stimulating hormone (FSH) were then measured 2 hours after the agonist administration by RIA. The oral administration was done through force-feeding with feeding tubes.

20

Table IV shows data on AOA and histamine release for analogs containing acylated aminocyclohexylalanine residues. For the analogs with NicLys<sup>5</sup>, D-NACAla<sup>6</sup>, IV-1 and IV-2, (NACAla represents 3(4-nicotinoyl-aminocyclohexyl)alanine), analog 2 with cis-D-NACAla<sup>6</sup> is somewhat more active, 100% vs. 70% AOA at  $\mu$ g. Analogs IV-7 and IV-8 with NicLys<sup>5</sup>, D-PzACAla<sup>6</sup> (PzACAla represents 3(4-pyrazinylcarbonylamino-cyclohexyl)alanine) show the opposite order of activity. The trans residue has the higher AOA, 88% vs. 25% at  $\mu$ g.

Analogs IV-3 and IV-4 with PicLys<sup>5</sup>, trans and cis PACAla<sup>6</sup> (PACAla represents 3(4-picolinoylaminocyclohexyl)alanine) are equipotent, 50 and 54% AOA at 0.5  $\mu$ g, respectively, whereas in the case of PicLys<sup>5</sup>, trans and cis PzACAla<sup>6</sup> the cis compound is more

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than twice as active. The former, analog IV-5 is about as potent as analogs IV-3 and IV-4 (44% at 0.5ug) while the latter, analog 6, has 100%, 73%, and 29% AOA at 0.5, 0.25, and 0.125ug, respectively. The high potency analog IV-6 is unique in comparison with the low activity of the structurally similar analog IV-8.

Analog IV-2 has cis-PzACala<sup>5</sup>, D-PicLys<sup>6</sup> and, although residues 5 and 6 are reversed, retained the high potency of analog IV-6, 90% and 67% at 0.5 and 0.25ug, respectively.

As for histamine release, all analogs tested, in vitro, have lower ED<sub>50</sub> values than the parent compounds. The ED<sub>50</sub> values range from about 30 to about 60 compared to >300 and 93±11 for Antide and analog V-10. The tests for wheal response show a range from 99.5 to 129.6, which is similar to Antide (132.7) and analog V-10 (123.0). The lack of correlation between the two tests may primarily reflect assay variation.

In summary, for the analogs with NicLys<sup>5</sup>, incorporation of aminocyclohexylalanine derivatives in position 6 resulted in substantial increase in, in vitro, histamine release and unchanged or lowered AOA. For the PicLys<sup>5</sup> analogs with the same substitutions there was lowering of AOA potency in all cases except one, where a considerable increase was observed. The combination PicLys<sup>5</sup> and cis-D-PzACala<sup>6</sup> evidently possesses some beneficial structure. Histamine release for the PicLys<sup>5</sup> analogs was increased by 50-100%.

In Table V, are the results from substitutions in position 7 of analog V-10. This position allows some structural freedom although none of the peptides show higher AOA than analog V-10. Analogs V-12, V-14, and V-16

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having Aile<sup>7</sup> (alloisoleucine), Val<sup>7</sup> and Abu<sup>7</sup> (2-aminobutyric acid), are equipotent with analog V-10. Analog V-16 with the straight chain Abu<sup>7</sup> is slightly more potent than analogs V-13 and V-15 with Nle<sup>7</sup> (norleucine) and Nval<sup>7</sup> (norvaline), respectively, which should more closely resemble the natural Leu<sup>7</sup>.

For compound V-17 with the small Ala<sup>7</sup>, the AOA decreased to 60% at 0.5ug. Incorporation of Trp<sup>7</sup> which is the natural residue in chicken II, salmon and lamprey LHRH's (13-15), gave analog 18 with only 10% AOA at 0.5ug. Trp<sup>7</sup> may be too large considering the size of the adjacent D-PicLys<sup>6</sup> and ILys<sup>8</sup>.

The most interesting feature of Table V is the, in vitro, histamine release data. The three analogs with similar AOA potency as analog V-10 show markedly diminished histamine release. The ED<sub>50</sub> values for analogs V-12, V-14, and V-16 are >300, 213±30 and 273±27, respectively; i.e., a 2-3 fold decrease in histamine release is achieved by small changes in side chain structure. Also, the wheal response is diminished for all analogs compared to V-10.

It was noted earlier (1) that whether ILys or IOrn is the best substituent in position 8 is sequence dependant. To further investigate this aspect, the IOrn<sup>8</sup> analogs corresponding to some of the best peptides were synthesized and tested. The results in Table VI indicate that ILys<sup>8</sup> may be better. For two of the pairs, analogs VI-10, VI-19 and VI-12, VI-21, ILys<sup>8</sup> and IOrn<sup>8</sup> were about equivalent. For the other three pairs, the analogs with ILys<sup>8</sup> were more active, but the differences were not large. The largest difference was for the pair with Val<sup>7</sup>, where the ILys<sup>8</sup>-analog VI-14 showed 90% AOA at 0.5ug vs. 57% for the IOrn<sup>8</sup>-analog VI-20.

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Analog VI-19 was tested, in vitro, for histamine release. The  $ED_{50}$  value is  $42 \pm 3.1$ ; i.e., the histamine release is 2-fold that of the analog with one more  $CH_2$  unit. The wheal response did not change conspicuously except for the Aile<sup>7</sup> and IOrn<sup>8</sup> analog 21 which had the low value of  $78.6 \pm 4.5$  compared to the ILys analog 12 which had  $97.9 \pm 2.9$ .

Table VII shows the duration of action of Antide and two analogs. When Antide was injected 44 hours before 50 ng of [D-Qal<sup>6</sup>]-LHRH (Qal represents 3(3-quinolyl)alanine), a superagonist, at doses of 3, 10, and 30ug, significant reductions in serum LH were observed at the two higher doses. The LH decreased from  $113 \pm 11$  to  $46 \pm 12$  and  $5 \pm 0.7$  ng/ul. Serum FSH was also decreased, most significantly from about 300 to about 300 ng/ul at 30ug.

Analog VII-24, [Tyr<sup>5</sup>]-Antide, and analog IV-6 were similarly injected 24 hours before the agonist. Analog VII-24 showed high activity, reducing the LH level to  $19 \pm 4$ ,  $3 \pm 0.4$  and  $0.3 \pm 0.03$  ng/ul at doses of 3, 10, and 30ug, respectively. The corresponding figures for analog IV-6 are  $42 \pm 7$ ,  $15 \pm 3$ , and  $3.4 \pm 2$  ng/ul. This is interesting since in the antioviulatory assay analog IV-6 is considerably more potent, 73% at 0.25 ug vs. 45% at 0.5 ug. Perhaps, analog IV-6 is enzymatically degraded faster than analog VII-24. The long duration of action of these analogs s.c. may also be due to "depot" effects at the site of injection.

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Table VIII shows the duration of action of Antide after oral administration. Forty-eight hours after administration of 100 or 300ug dose levels of Antide, there were significantly reduced levels of LH which had been released by 5 ng of [D-Qal<sup>6</sup>]-LHRH s.c. Reductions from  $21 \pm 3$  to  $4 \pm 0.8$  and  $8 \pm 2$  ng/ul, respectively, were

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observed. The results are about the same in the -24 hour experiment ( $9 \pm 2$  and  $6 \pm 0.3$  ng/ul). Antide appears to possess considerable resistance towards degrading enzymes. When Antide was given 2 hours before the agonist, a strong decrease in LH levels was observed. At a dose of 30ug, a significant lowering of the LH level to  $6 \pm 1$  ng/ul was seen. At 100 and 300ug, the levels were  $1 \pm 0.3$  and  $0.4 \pm .4$  ng/ul, i.e., very low levels. When 10 ng of agonist was used, the results are qualitatively very similar.

10

For comparison, the last three entries in Table VIII are from experiments with [N-Ac-D-pClPhe<sup>1,2</sup>,D-Trp<sup>3</sup>,D-Arg<sup>6</sup>,D-Ala<sup>10</sup>]-LHRH, VIII-25, an analog that has been reported to have oral activity, (16). These data show that Antide is more active than VIII-25, since a dose of 30ug given 2 hours before the agonist reduced the LH level from  $44 \pm 4$  to  $22 \pm 4$  ng/ul ( $p < 0.01$ ). The value for analog VIII-25 is  $39 \pm 6$  (NS). At 100 ug, the corresponding numbers are  $7 \pm 3$  ( $p < 0.001$ ) and  $26 \pm 7$  ( $p < 0.05$ ). The FSH levels were, in general, lowered when Antide was administered at -2 hours at 100 or 300ug dose levels.

The results in Table IX show that there is no significant difference between administration of Antide in water or in corn oil.

Antide has also been tested orally in the antiovaratory assay (Table X). The AOA values at 300, 600, and 1200ug dose levels are 18, 73, and 100% respectively. Expressed as rats ovulated/total rats, the numbers are 9/11, 3/11, and 0/11. For analog VIII-25, the numbers 9/11, 4/11, and 0/11 have been reported at dose levels of 500, 1000, and 2000ug, respectively, (16). Antide was about twice as active as analog VIII-25.

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Table XI shows a comparison of the oral activities of Antide and four analogs. One was as active as Antide, one was considerably less active and two were less active at low doses (30 and 100ug) and about as active at 300ug.

5

After a 15 ng s.c. dose of [D-Qal<sup>6</sup>]-LHRH, the LH level rose to  $91 \pm 4.6$  ng/ul. At oral dose levels of 30, 100, and 300ug of Antide, reduced levels of LH of  $75 \pm 3$ ,  $20 \pm 4$ , and  $5 \pm 1$  ng/ul, respectively, were recorded. Analog 4 with PicLys<sup>5</sup>, and D-PACala<sup>6</sup> showed no significant reduction of LH at 30 and 100ug levels, but there was a reduction to  $51 \pm 6$  ng/ul at a 300ug dose.

15 Analog V-12 with PicLys<sup>5</sup>, D-PicLys<sup>6</sup>, and Aile<sup>7</sup> and analog IV-6 with PicLys<sup>5</sup>, cis-D-PzACala<sup>6</sup> are less active than Antide at 30 and 100ug, but were equally active at 300 ug. Both of these peptides were substantially more active than Antide in the s.c. antiovulatory assay.

20 Analog 26 was equipotent with Antide. This is not suprising since the only structural difference between these analogs is a pyrazine instead of a pyridine moiety in the N<sup>E</sup>-acyl group of the D-Lys<sup>6</sup> residue.

25 Table XI and XII also shows results with Antide, for example, when 50 ng of the agonist was used. Comparison of these results with the data from the experiments using 15 ng of agonist shows a dose-response relationship which is expected from competitive antagonism. Using 15 ng of 30 agonist, 100 and 300ug of Antide reduced the LH level from  $115 \pm 15$  ng/ul to  $20 \pm 4$  and  $5 \pm 1$  ng/ul respectively, while in the experiments using 50 ng of agonist, 300 and 900ug of Antide reduced the LH to the same level ( $19 \pm 3$  and  $5.3 \pm 1.2$  ng/ul).

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Table XIII shows the biological effects of Antide in a dispersed pituitary cell culture system.

The structures and biological activities of certain preferred LHRH analogs inhibiting more than 50% of ovulatory activity at a dose of 0.25 ug are shown in Table XIV.

It is proposed that Antide and other antagonists of the present invention may be utilized to induce a state of reversible medical castration that will be of value in the treatment of a rather large number of diseased states such as endometriosis, uterine fibroids and hormonal dependent cancers (prostate, breast). In some patients temporary inhibition of the function of the gonads with Antide, for example, while the patient is receiving chemotherapeutic agents and/or irradiation may prevent or minimize adverse effects of these agents on the gonads and thus help to preserve future fertility. Therapeutic examples would be irradiation during bone marrow transplantation, cervical carcinoma, metastatic thyroid and uterine carcinoma, possibly thyrotoxicosis, etc. during chemotherapy for disseminated lupus erythematosus and certain stages of organ transplantation. More physiological usages of the antagonists of the present invention such as Antide would be to inhibit fertility in both females and males.

More unique possible usages of Antide or other decapeptides of the present invention would be to modify sexual behavior during select disease states. Such disease states could involve patients with AIDS, the aggressive behavior of sex offenders in prisons or aggressive adolescents confined to corrective institutions. It is also possible that high serum gonadotrophin levels of post-menopausal women may induce functional abnormalities in fat cells that cause weight



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gain or in bone cells that play a role in accelerated osteoporosis. These functional abnormalities could potentially be reduced with administration of Antide by inhibiting the high LH and/or FSH level in serum of post  
5 menopausal women.

Selective LH-RH antagonists mainly with charged amino acid substitutions in position 6 and/or 8 of the decapeptides probably stimulate histamine release by a  
10 direct effect on mast cells to release histamine while other LH-RH antagonists like Antide do not. It is thus proposed that the mast cell-stimulating antagonists applied locally to wounds of the skin may accelerate healing while non-histamine stimulating antagonists may  
15 prevent some of the allergic reactions which occur in humans.

To delay the onset of puberty in short stature children by administration of Antide with and without  
20 concomitant administration of GH or GH-releasing peptides is proposed as a unique method to enhance body height. The presence of gonadal hormones fuse the epiphysis of long bone and prevent their further elongation. This approach should extend and augment the use and  
25 effectiveness of GH and GH-releasing peptides.

The administration of LH-RH antagonists of the present invention acutely inhibits the function of the gonads within 24 hours. Continuous administration of LH-  
30 RH superagonists also inhibits the function of the gonads but this is only after several days of stimulating the gonads to hyperfunction. Such superagonist administration introduces a number of potential undesirable clinical problems in patients with prostate cancer, endometriosis,  
35 uterine fibroids as well as with sex offenders and those subjected to a temporary induction of medical castration.

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For these reasons it is proposed that LH-RH antagonists will be more desirable agents than LH-RH agonists for introducing a reversible state of medical castration. At the diagnostic level, such as differentiating the anatomic source of steroid secretion from the adrenal versus the ovary or to reveal the degree of calcium excretion dependency on gonadal steroid hormones, the rapid onset of inhibiting gonadal function with LH-RH antagonists makes them an unequivocally superior agent over LH-RH agonists.

It is proposed that, in every clinical situation where LH-RH superagonists have been utilized to inhibit gonadal function, the LH-RH antagonists will be the agents of choice.

The references in the following list are incorporated by reference herein.

1. Ljungqvist, A., Feng, D.-M., Tang, P.-F.L., Kubota, M., Okamoto, T., Zhang, Y., Bowers, C.Y., Hook, W.A. & Folkers K. (1987) Biochem. Biophys. Res. Commun. 148 (2), 849-586.
2. Karten, M.D. & Rivier, J.E. (1986) Endocr. Rev. 7, 44-56.
3. Hook, W.A., Karten, M. & Siraganian, R. P. (1985) Fed. Proc. Fed. Am. Soc. Exptl. Biol. 44, 1323.
4. Karten, M.D., Hook, W.A., Siraganian, R.P., Coy, D.H., Folkers, K., Rivier, J.E. & Roeske, R.W. (1987) in LHRH and its Analogs; Contraceptive and Therapeutic Applications Part 2, eds. Vickery, B.H. & Nestor, J.J., Jr., (MTP Press Ltd., Lancaster, England) pp. 179-190.

**SUBSTITUTE SHEET**

5. Rivier, J.E., Porter, J., Rivier, C.L., Perrin, M., Corrigan, A., Hook, W.A., Siraganian, R.P. & Vale, W.W. (1986) J. Med. Chem. 29, 1846-1851.
- 5 6. Roeske, R.W., Chaturvedi, N.C., Hrinyo-Pavlina, T., & Kowalczyk, M. (1987) in LHRH and its Analogs; Contraceptive and Therapeutic Applications Part 2, eds. Vickery, B.H. & Nestor, J.J., Jr., (MTP Press Ltd., Lancaster, England) pp. 17-24.
- 10 7. Hocart, S.J., Nekola, M.V. & Coy, D.H. (1987) J. Med. Chem. 30, 739-743.
8. Nestor, J.J., Tahilramani, R., Ho, T.L., McRae, G.I. & Vickery, B.H. (1988) J. Med. Chem. 31, 65-72.
- 15 9. Bajusz, S., Kovacs, M., Gazdag, M., Bokser, L., Karashima, T., Csernus, V.J., Janaky, T., Guoth, J. & Schally, A.V. (1988) Proc. Natl. Acad. Sci. USA 85, 1637-1641.
- 20 10. Rivier, J., Kupryszewski, G., Varga, J., Porter, J., Rivier, C., Perrin, M., Hagler, A., Struthers, S., Corrigan, A. & Vale, W. (1988) J. Med. Chem. 31, 677-682.
- 25 11. Folkers, K., Bowers, C.Y., Shieh, H.-M., Liu, Y.-Z., Xiao, S.-B., Tang, P.-F.L. & Chu, J.-Y. (1984) Biochem. Biophys. Res. Commun. 123 (3) 1221-1226.
- 30 12. Humphries, J., Wan, Y.-P., Folkers, K. & Bowers, C.Y. (1978) J. Med. Chem. 21(1), 120-123.
13. Miyamoto, K., Hasegawa, Y., Nomura, M., Igarashi, M., Kanagawa, K. & Matsuo, H. (1984) Proc. Natl. Acad. Sci. USA 81, 3874-3878.
- 35

**SUBSTITUTE SHEET**

14. Sherwood, N., Eiden, L., Brownstein, M., Spiess, J., Rivier, J., & Vale, W. (1983) Proc. Natl. Acad. Sci. USA 80, 2794-2798.
- 5 15. Sherwood, N.M., Sower, S.A., Marshak, D.R., Fraser, B.A. & Brownstein, M.J. (1986) J. Biol. Chem. 261, 4812-4819.
16. Nekola, M.V., Horvath, A., Ge, L.-J., Coy, D.H. & Schally, A.V. (1982) Science 218, 160-161.
- 10 17. Bernardi, et al., J. Pharm. Pharmacol. 19, 95 (1967).
18. Fife, T.H. and Przystas, T.J., J. Am. Chem. Soc. 107, 1041 (1985).
- 15 19. Lecher et al., U.S. 2,872,484, Feb. 3, 1959, Chem. Abstr. 53, 11238c.
- 20 20. Tjoeng et al., Chem. Ber. 108, 862 (1975).
21. Humphries et al., J. Med. Chem. 21(1), 120 (1978).
22. Benoiton, L., Can. J., Chem. 42, 2043 (1969).
- 25 23. Prasad et al., J. Med. Chem. 19, 492 (1976).
24. Zinner, H. and Fiedler, H., Arch. Pharm. 291(63), 330 (1958).
- 30
- Changes may be made in the particular amino acid or derivatives and their assembly described herein or in the steps or the sequence of steps of the method described herein without departing from the concept and scope of the invention as defined in the following claims.
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CLAIMS:

1. A decapeptide having antioviulatory activity  
5 comprising Ser<sup>4</sup>, PicLys<sup>5</sup> and D-PicLys<sup>6</sup>.
2. A decapeptide having antioviulatory activity  
comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, Ser<sup>4</sup>, D-PicLys<sup>5</sup> and  
10 Pro<sup>9</sup>.
3. A decapeptide having antioviulatory activity  
comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, D-  
15 PicLys<sup>6</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>.
4. A decapeptide having antioviulatory activity  
comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>,  
20 NicLys<sup>5</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>.
5. A decapeptide having antioviulatory activity  
comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, Leu<sup>7</sup>,  
25 Pro<sup>9</sup> and D-Ala<sup>10</sup>.
6. A decapeptide having antioviulatory activity  
comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, Leu<sup>7</sup>,  
30 Pro<sup>9</sup> and D-Ser<sup>10</sup>.
7. A decapeptide having antioviulatory activity  
comprising D-pClPhe<sup>2</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>.

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8. A decapeptide having antioviulatory activity comprising D-pClPhe<sup>2</sup>, Pro<sup>9</sup> and Ser<sup>10</sup>.
- 5 9. A decapeptide having antioviulatory activity comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, ILys<sup>8</sup> and D-Ala<sup>10</sup>.
- 10 10. A decapeptide having antioviulatory activity comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, ILys<sup>8</sup> and D-Ala<sup>10</sup>.
- 15 11. A decapeptide having antioviulatory activity comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, PicLys<sup>5</sup>, D-PicLys<sup>6</sup>, ILys<sup>8</sup> and D-Ala<sup>10</sup>.
- 20 12. A decapeptide having antioviulatory activity comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, IOrn<sup>8</sup> and D-Ala<sup>10</sup>.
- 25 13. A decapeptide having antioviulatory activity comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, PicLys<sup>5</sup>, D-PicLys<sup>6</sup>, IOrn<sup>8</sup> and D-Ala<sup>10</sup>.
- 30 14. A decapeptide having antioviulatory activity comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, MNicLys<sup>5</sup>, D-MNicLys<sup>6</sup>, IOrn<sup>8</sup> and D-Ala<sup>10</sup>.

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15. A decapeptide having antioviulatory activity comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, PzcLys<sup>5</sup>, D-PzcLys<sup>6</sup>, IOrn<sup>8</sup> and D-Ala<sup>10</sup>.
- 5
16. A decapeptide having antioviulatory activity comprising N-Ac-D-pClPhe<sup>1</sup>, D-3-Pal<sup>3</sup>, Tyr<sup>5</sup>, D-NicLys<sup>6</sup> and ILys<sup>8</sup>.
- 10
17. A decapeptide having antioviulatory activity comprising N-Ac-D-Cl<sub>2</sub>Phe<sup>1</sup>, D-3-Pal<sup>3</sup>, Tyr<sup>5</sup>, D-NicLys<sup>6</sup> and ILys<sup>8</sup>.
- 15
18. A decapeptide having antioviulatory activity comprising acylated Lys<sup>5</sup>, D-acylated Lys<sup>6</sup> and N-alkylated diamino acid<sup>8</sup>.
- 20
19. A decapeptide having antioviulatory activity comprising NicLys<sup>5</sup>, D-NicLys<sup>6</sup> and ILys<sup>8</sup>.
- 25
20. A decapeptide having antioviulatory activity comprising PicLys<sup>5</sup>, D-PicLys<sup>6</sup> and ILys<sup>8</sup>.
- 30
21. A decapeptide having antioviulatory activity comprising NicLys<sup>5</sup>, D-NicLys<sup>6</sup> and IOrn<sup>8</sup>.
- 35
22. A decapeptide having antioviulatory activity comprising PicLys<sup>5</sup>, D-PicLys<sup>6</sup> and IOrn<sup>8</sup>.

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23. A decapeptide having antioviulatory activity comprising MNicLys<sup>5</sup>, D-MNicLys<sup>6</sup> and IOrn<sup>8</sup>.
- 5 24. A decapeptide having antioviulatory activity comprising PzcLys<sup>5</sup>, D-PzcLys<sup>6</sup> and IOrn<sup>8</sup>.
- 10 25. A decapeptide having antioviulatory activity comprising Tyr<sup>5</sup>, D-NicLys<sup>6</sup> and ILys<sup>8</sup>.
- 15 26. A decapeptide having antioviulatory activity comprising Tyr<sup>5</sup>, D-NicLys<sup>6</sup> and IOrn<sup>8</sup>.
- 20 27. A decapeptide having antioviulatory activity comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.
- 25 28. A decapeptide having antioviulatory activity comprising N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, PicLys<sup>5</sup>, cis D-PzACAla<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.
- 30 29. A process for inhibiting ovulation in an animal comprising administering to said animal a decapeptide having the structure: N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.
- 35 30. A process for inhibiting ovulation in an animal comprising administering to said animal a decapeptide having the structure: N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>,

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Ser<sup>4</sup>, PicLys<sup>5</sup>, cis D-PzACAla<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.

5 31. A process for inhibiting the onset of puberty in an animal comprising administering to said animal a decapeptide having the structure: N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.

10

32. A process for inhibiting the sexual impetus of an animal comprising administering to said animal a decapeptide having the structure: N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.

15

33. A process for altering the gonadal function of an animal comprising administering to said animal a decapeptide having the structure: N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.

20

25 34. A process for inhibiting the growth of hormone-dependent tumors in an animal comprising administering to said animal a decapeptide having the structure: N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, Leu<sup>7</sup>, ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.

30

35. A process for lowering LH and FSH levels in serum of post-menopausal woman comprising administering to said woman a decapeptide having the structure: N-Ac-D-2-Nal<sup>1</sup>,

35

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D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, Ser<sup>4</sup>, NicLys<sup>5</sup>, D-NicLys<sup>6</sup>, Leu<sup>7</sup>,  
ILys<sup>8</sup>, Pro<sup>9</sup> and D-Ala<sup>10</sup>NH<sub>2</sub>.

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TABLE I. ANTAGONISTS OF LHRH BASED UPON

$$[( )^1, D\text{-pClPhe}^2, ( )^3, \text{Ser}^4, ( )^5, ( )^6, \text{Leu}^7, ( )^8, \text{Pro}^9, D\text{-Ala}^{10}] \text{-NH}_2$$

NO.	IBR #	( ) <sup>1</sup>	( ) <sup>3</sup>	Compound ( ) <sup>5</sup>	( ) <sup>6</sup>	( ) <sup>8</sup>	AOA %/μg 1.0 2.0	Wheal Area mm <sup>2</sup> /10μg	E <sub>50</sub> μg/ml
ANALOGS WITH D-NICLYS IN POSITION 6									
1.	22396	N-Ac-D-2-Nal	D-3-Pal	Tyr	D-NicLys	Arg	60	85	39.2±7
2.	24753	"	"	"	"	Me <sub>3</sub> Arg	--	--	39.9±7
3.	24825	"	"	"	"	Lys	--	119.5±3.2	133±22
4.	24315	"	"	"	"	ILys	45	79.0±9.2	18.4
5.	24443	"	"	"	"	Me <sub>2</sub> Lys	--	122.7	19.3
6.	24748	"	"	"	"	Orn	--	129.4±3.3	>300
7.	24756	"	"	"	"	IOrn	22	92.2±2.9	1.73
8.	24199	"	"	Arg	"	Arg	0	146.8	4.3±0.52
9.	24446	"	D-Tyr	"	"	"	33	113.2±5.6	20.3
10.	25335	"	D-3-Pal	"	"	ILys	43	196.9±4.2	
11.	24931	"	"	Me <sub>3</sub> Arg	"	"	--	140±7.0	
12.	25506	"	"	Dpo	"	"	56	110±3	
13.	24543	"	"	ILys	"	"	--	132.7±0	
14.	24545	"	"	His	"	Arg	--	139.7±0	
15.	24593	"	"	3-Pal	"	"	--	146.4±3.6	
16.	25383	"	"	"	"	ILys	--	132.8±6.0	86±28*
17.	25384	"	"	"	"	IOrn	100	139.9±7.2	55±13*
18.	25144	"	"	Ile	"	ILys	--	147.7±7.1	324±20
19.	25145	"	"	"	"	IOrn	--	116.5±8.7	151±75
20.	25333	"	"	NicOrn	"	"	--	113.6±10.9	57±13
21.	25509	"	"	DMGlys	"	ILys	20	110±3	34±1.1
22.	25510	"	"	PicLys	"	"	64	116±3.3	39±1.0
23.	25337	N-Ac-D-pClPhe	"	Tyr	"	"	--	139.9±7.2	198±33*
24.	25338	N-Ac-D-Cl <sub>2</sub> Phe	"	"	"	"	0	103.9±5.3	311±65*

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## ANALOGS WITH NICLYS IN POSITION 5

	N-Ac-D-2-Nal	D-3-Pal	NicLys	D-3-Pal	Arg	0			
25.	22495	"	"	D-His	"	--	--	112	
26.	24544	"	"	D-ILys	"	--	100	146.7±3.6	
27.	24754	"	"	D-Dpo	ILys	40	56	196.9±4.1	
28.	25334	"	"	D-BzLys	"	--	100	165.2±6.7	6.7±2.2
29.	25332	"	"	D-Et <sub>2</sub> hArg	"	--	50	119.6±6.7	>300
30.	25507	"	"	D-Pi <sub>2</sub> CLys	"	--	67	123±5.8	
31.	25589	"	"	D-An <sub>2</sub> GLU	"	36	--	120±7	60±1.4
32.	25588	"	"	trans-D-NACALA	"	--	67	113±7	>300
33.	25647	"	"	cis-D-NACALA	"	--	70	119.5±3.2	
34.	25648	"	"	D-Me <sub>2</sub> Lys	"	--	100	113.6±10.9	37±1.1
35.	25591	"	"	D-Pz <sub>2</sub> CLys	"	--	82	111±2	262±23
36.	25649	"	"	"	"	78	92	122.2±5.1	

## ANALOGS WITH NICLYS IN POSITION 8

	N-Ac-D-2-Nal	D-3-Pal	Tyr	D-Arg	NicLys	--			
37.	24749	"	Arg	D-3-Pal	"	--	--	136.3±6.8	14.2
38.	24771	"	Tyr	D-ILys	"	--	--	99.0±10.3	
39.	24824	"	"	"	"	--	100	122.8±5.8	

## ANALOGS WITH NICLYS AND D-NICLYS

## IN POSITIONS 5, 6 OR IN POSITION 8, 6 OR IN POSITIONS 3, 6

	N-Ac-D-2-Nal	D-3-Pal	NicLys	D-NicLys	Arg	22			
40.	24594	"	"	"	Me <sub>3</sub> Arg	--	100	126.2±8.8	
41.	24987	"	"	"	Dpo	--	100	150.9±14.0	
42.	25143	"	"	"	ILys	36	18	113.6±11.1	<300
43.	24542	"	"	"	IOrn	88	100	132.7±0	300
44.	24933	"	"	"	CyPLys	--	64	136.0±3.4	206±64
45.	25078	"	"	"	NicLys	--	0	147.0±7.1	171±49
46.	24540	"	Tyr	"	"	--	--	82.6±2.8	300
47.	24745	"	His	"	"	--	18	136.3±6.8	
48.	24746	"	ILys	"	"	--	30	132.8±5.9	
49.	24597	"	D-NicLys	"	Arg	--	89	101.0±6.0	

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## MISCELLANEOUS ANALOGS

NO.	IBR #	( ) <sup>1</sup>	Compounds ( ) <sup>3</sup> ( ) <sup>5</sup>	( ) <sup>6</sup> ( ) <sup>8</sup>	AOA 0.5 1.0	%/μg 2.0	10.0	Whgal Area mm <sup>2</sup> /10μg	E <sub>D</sub> 50 μg/ml
50.	24596	N-Ac-D-2-Nal	D-3-Pal, NicLys,	D-NicLys, NicLys	--	0	--	122.8±5.7	--
51.	24934	"	"	NicLys Ilys	--	--	8	123±5.9	>300
52.	25146	"	INicLys	D-INicLys "	--	63 91	--	140.3±13.9	15±8.2
53.	25147	"	PicLys	D-PicLys "	40	100 90	--	123.0±0	93±28
54.	25385	"	Arg	D-BzLys "	--	63	--	169.0±7.7	8.7±3*
55.	25386	"	MNcLys	D-MNcLys "	--	56 100	--	126.1±6.7	>300*
56.	25508	"	DMGLys	D-BzLys "	--	100	--	136.6.7	24±0.3
57.	25650	"	PzCLys	D-PzCLys "	--	17	--	110.2±8.1	288±30

\*In this test series, the standard compound had an E<sub>D</sub> 50 value of 0.46 instead of the usual 0.1 -0.2.

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TABLE II  
ANALOGS WITH PicLys<sup>5</sup>, D-PicLys<sup>6</sup>

IBR #	Sequence									
	N-Ac-D-Cl <sub>2</sub> Phe, N-Ac-D-2-Nal	D-pClPhe, D-Cl <sub>2</sub> Phe D-3-Pal	D-3-Pal, D-pClPhe D-3-PzAla D-Trp D-3-Pal	Ser, "	PicLys, "	D-PicLys, "	Leu, "	ILys, "	Pro, "	D-Ala-NH <sub>2</sub> "
58.	26100									
59.	25807									
60.	26364							IOrn		
61.	26119							"		
62.	26177							ILys		
63.	25934						Val	"		
64.	26118						"	IOrn		
65.	25936						Aile,	ILys		
66.	26178						"	IOrn		
67.	25990						Abu	ILys		
68.	26179						"	IOrn		
69.	25935						Trp	ILys		
70.	25988						Nle	"		
71.	25989						Nval	"		
72.	26020						Ile	"		
73.	26099						Ala	"		
74.	26346						Abu	Arg		
75.	25937						Leu	ILys		
76.	26019						"	"	Pip	Pro, D-Abu-NH <sub>2</sub>
77.	25933						"	IOrn	"	D-Ala-NH <sub>2</sub>

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Analogues with PicLys<sup>5</sup>

		N-Ac-D-2-Nal	D-pClPhe	D-TinGly	Ser	PicLys	ε-D-PzACAla	Leu	ILys	Pro	D-Ala-NH <sub>2</sub>
78.	26349	"	"	D-3-PxAla	"	"	"	"	"	"	"
79.	26324	"	"	D-3-Pal	"	"	"	"	"	"	"
80.	25897	"	"	"	"	"	"	"	"	"	"
81.	26181	"	"	"	"	"	"	"	IOrn	"	"
82.	26325	"	"	"	"	"	"	Val	ILys	"	"
83.	26366	"	"	"	"	"	"	Phe	"	"	"
84.	26347	"	"	"	"	"	"	Leu	Arg	"	"
85.	26348	"	"	"	"	"	"	"	ILys	"	D-Ser-NH <sub>2</sub>
86.	26383	"	"	"	"	"	"	"	"	"	NHET
87.	26323	"	"	"	"	"	ε-D-PmACAla	"	"	"	D-Ala-NH <sub>2</sub>

Analogues with D-PicLys<sup>6</sup>

		N-Ac-D-2-Nal	D-pClPhe	D-3-Pal	Ser	ε-PzACAla	D-PicLys	Leu	ILys	Pro	D-Ala-NH <sub>2</sub>
88.	26180	"	"	"	"	HOBLys	"	Abu	"	"	"
89.	26381	"	"	"	"	Cit	"	"	"	"	"
90.	26382	"	"	"	"	Tyr	"	Leu	IOrn	"	"
91.	26363	"	"	"	"	"	"	"	"	"	"

Analogues with NicLys<sup>5</sup>

		N-Ac-D-2-Nal	D-pClPhe	D-3-Pal	Ser	NicLys	ε-D-PzACAla	Leu	ILys	Pro	D-Ala-NH <sub>2</sub>
92.	25805	"	"	"	"	"	ε-D-PzACAla	"	"	"	"
93.	25806	"	"	"	"	"	D-NicLys	NMeLeu	"	"	"
94.	26345	"	"	"	"	"	D-PzcLys	Leu	IOrn	"	"
95.	25991	"	"	"	"	"	"	"	"	"	"

## Miscellaneous Substitutions in Positions 5 and 6.

	25808	N-Ac-D-2-Nal	D-pClPhe	D-3-Pal	Ser	MPicLys	D-M-PicLys	Leu	ILys	Pro	D-Ala-NH <sub>2</sub>
96.	26322	"	"	"	"	PmcLys	D-PmcLys	"	"	"	"
97.	26326	"	"	"	"	ε-PzACAla	ε-D-PzACAla	"	"	"	"
98.	26417	"	"	"	"	"	D-3-PzAla	"	Arg	"	"
99.	26418	"	"	"	"	Tyr	ε-D-PzACAla	"	ILys	"	"
100.	26365	"	"	"	"	"	"	"	"	"	"
101.											D-Ser-NH <sub>2</sub>

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## Analogues Being Synthesized at This Time.

102.	N-Ac-D-2-Nal	D-pClPhe	D-3-Pal	Ser	Arg	D-3-Pal	Leu	Arg	Pro	Sar-NH <sub>2</sub>
103.	"	"	"	"	PicLys	$\epsilon$ -D-PzACAla	"	ILys	"	"
104.	D-pGlu	"	D-Phe	"	Arg	D-3-Pal	"	Arg	"	D-Ala-NH <sub>2</sub>
105.	N-Ac-D-2-Nal	"	D-3-Pal	"	$\epsilon$ -PzACAla	D-PicLys	Val	ILys	"	"



TABLE III Biological Data.  
Analogues with picLys<sup>5</sup>, D-PicLys<sup>6</sup>

NO.	IBR #	AOA/ $\mu$ g			Wheal Area mm <sup>2</sup> /10 $\mu$ g	In Vitro	
		0.25	0.5	1.0		Histamine Release ED <sub>50</sub> $\mu$ g/ml $\pm$ SEM	
58.	26100	-	38	-	116.2 $\pm$ 3.7		
59.	25807	-	64	90	139.8 $\pm$ 7.1		
60.	26364	12	-	-	116.2 $\pm$ 5.5		
61.	26119	-	75	-	103.9 $\pm$ 3.4		
62.	26177	-	20	-	71.0 $\pm$ 4.3		
63.	25934	43	90	100	97.9 $\pm$ 2.9	213 $\pm$ 30	
64.	26118	-	57	-	119.6 $\pm$ 6.6		
65.	25936	43	89	-	97.9 $\pm$ 2.9	> 300	
66.	26178	-	82	-	78.6 $\pm$ 4.5		
67.	25990	36	100	-	91.0 $\pm$ 5.4	273 $\pm$ 27	
68.	26179	-	80	-	101.5 $\pm$ 9.3		
69.	25935	-	10	-	78.5 $\pm$ 0		
70.	25988	20	77	-	107.0 $\pm$ 6.0		
71.	25989	10	100	-	95.3 $\pm$ 6.0		
72.	26020	0	-	-	110.7 $\pm$ 2.3		
73.	26099	-	60	-	103.9 $\pm$ 3.7		
74.	26346	50	88	-	113.2 $\pm$ 5.4		
75.	25937	-	0	100	95.0 $\pm$ 0		
76.	26019	-	78	-	109.9 $\pm$ 3.0		
77.	25933	50	90	100	113.0 $\pm$ 0		

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Analogues With PicLys<sup>5</sup>

78.	26349	0	-	-	84.6±3.9
79.	26324	22	100	-	127.8±4.9
80.	25897	73	100	-	122.8±5.7
81.	26181	50	100	-	101.6±2.2
82.	26325	73	100	-	127.8±4.9
83.	26366	0	-	-	116.2±3.2
84.	26347	14	-	-	119.6±8.5
85.	26348	22	-	-	122.8±5.7
86.	26383	25	-	-	119.6±6.6
87.	26323	-	9	-	120.4±4.7

28 ± 7.5

Analogues With D-PicLys<sup>6</sup>

88.	26180	67	90	-	99.5±4.5
89.	26381	11	-	-	95.1±5.0
90.	26382	11	-	-	89.5±5.5
91.	26363	0	-	-	113.2±5.5

Analogues With NicLys<sup>5</sup>

92.	25805	-	67	88	129.6±8.8
93.	25806	-	-	25	101.7±5.0
94.	26345	10	-	-	110.5±11.4
95.	25991	-	44	-	104.3±10.5

## Analogues With Miscellaneous Substituents in Positions 5 and 6.

96.	25808	-	67	91	106.2±4.3
97.	26322	0	-	-	130.2±2.5
98.	26326	57	100	-	115.5±2.4
99.	26417	22	-	-	133.2±11.8
100.	26418	22	-	-	95.0±0
101.	26365	0	-	-	129.4±3.3

TABLE IV

Biological Data for [N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, X<sup>5</sup>, Y<sup>6</sup>, Ilys<sup>8</sup>, D-Ala<sup>10</sup>]-LHRH Analogs

NO.	X	Y	AOA %/µg			In Vitro Histamine Release ED <sub>50</sub> µg/ml±SEM	Wheal Area mm <sup>2</sup> /10µg
			0.125	0.25	0.5	1.0	
IV-1.	NicLys	trans-D-NACala	-	-	-	70	119.5±3.2
IV-2.	"	cis-D-NACala	-	-	50	100	101.8±4.3
IV-3.	PicLys	trans-D-PACala	-	-	50	-	101.0±3.0
IV-4.	"	cis-D-PACala	-	-	54	-	123.0±5.0
IV-5.	"	trans-D-PzACala	-	-	44	-	106.3±4.3
IV-6.	"	cis-D-PzACala	29	73	100	-	122.8±5.7
IV-7.	NicLys	trans-D-PzACala	-	-	67	88	129.6±8.8
IV-8.	"	cis-D-PzACala	-	-	-	25	101.7±5.0
IV-9.	cis-PzACala	D-PicLys	-	67	90	-	99.5±4.5

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TABLE V

Biological Data for [N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, PicLys<sup>5</sup>, D-PicLys<sup>6</sup>, X<sup>7</sup>, ILys<sup>8</sup>, D-Ala<sup>10</sup>]-LHRH Analogs.

NO.	X	AOA %/μg		In Vitro Histamine Release		Whegl Area mm <sup>2</sup> /10μg
		0.25	1.0	ED <sub>50</sub> μg/ml±SEM		
V-10.*	Leu	40	100	93±11		123±0
V-11:	Ile	0	-			110.7±2.3
V-12.	Aile	43	89	>300		97.9±2.9
V-13.	Nle	20	77			107.0±6.0
V-14.	Val	43	90	213±30		97.9±2.9
V-15.	NVal	10	100			95.3±6.0
V-16.	Abu	36	100	273±27		91.0±5.4
V-17.	Ala	-	60			103.9±3.7
V-18.	Trp	-	10			78.5±0

\* From Reference 1

TABLE VI

Biological Data for [N-Ac-D-2-Nal<sup>1</sup>, D-pClPhe<sup>2</sup>, D-3-Pal<sup>3</sup>, PicLys<sup>5</sup>, X<sup>6</sup>, Y<sup>7</sup>, Z<sup>8</sup>, D-Ala<sup>10</sup>]-LHRH Analogs

NO.	X	Y	Z	AOA <sub>50</sub> /μg		In Vitro Histamine Release ED <sub>50</sub> μg/ml±SEM	Wheal Area mm <sup>2</sup> /10μg
				0.25	1.0		
VI-10.*	D-PicLys	Leu	ILys	40	100	93±11	123±0
VI-19.	"	"	IOrn	50	90	42±3.1	113.0±0
VI-14.	"	Val	ILys	43	90	213±30	97.9±2.9
VI-20.	"	"	IOrn	-	57	-	119.6±6.6
VI-12.	"	Aile	ILys	43	89	>300	97.9±2.9
VI-21	"	"	IOrn	-	82	-	78.6±4.5
VI-16.	"	Abu	ILys	36	100	273±27	91.0±5.4
VI-22.	"	"	IOrn	-	80	-	101.5±9.3
VI-6. cis-D-PzACAla		Leu	ILys	73	100	28±7.5	122.8±5.7
VI-23.	"	"	IOrn	50	100	-	101.6±2.2

\* From Reference 1

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TABLE VII

Duration of Action of Antide and Two Analogs Subcutaneously\* Administered.

Analog	Injection Time	Dose $\mu$ g	0 Time ng sc [D-3-Oal]- LHRH	+2 hrs		
				LH ng/ml $\pm$ SEM	p value	FSH ng/ml $\pm$ SEM
-	-	-	-	0.4 $\pm$ 0.03	<.001	143 $\pm$ 10
-	-	-	50	113 $\pm$ 11	-	2899 $\pm$ 387
Antide	-44hr	3	50	90 $\pm$ 5	NS	2497 $\pm$ 155
"	"	10	50	46 $\pm$ 12	<.001	1413 $\pm$ 230
"	"	30	50	5 $\pm$ 0.7	<.001	311 $\pm$ 34
VII-24†	-24hr	3	50	19 $\pm$ 4	<.001	719 $\pm$ 123
"	"	10	50	3 $\pm$ 0.4	<.001	289 $\pm$ 30
"	"	30	50	0.3 $\pm$ 0.03	<.001	147 $\pm$ 10
IV-6(25897)	"	1	50	91 $\pm$ 19	NS	2020 $\pm$ 295
"	"	3	50	42 $\pm$ 7	<.001	1298 $\pm$ 275
"	"	10	50	15 $\pm$ 3	<.001	624 $\pm$ 84

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Analog	Injection Time	Dose $\mu$ g	0 Time ng SC <sub>6</sub> [D-3-Qal <sup>6</sup> ]- LHRH	+2 hrs	
				LH ng/ml $\pm$ SEM	FSH ng/ml $\pm$ SEM
"	"	30	50	3.4 $\pm$ 2	273 $\pm$ 89
				p value	p value
				<.001	<.001

\* Mean of 6  $\pm$  SEM† [Tyr<sup>5</sup>]-Antide

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TABLE VIII

Duration of Action of Orally Administered Antide and Comparison with

[N-Ac-D-pClpHe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Arg<sup>6</sup>, D-Ala<sup>10</sup>]-LHRH (25).\*

Antagonist	Time of adm.†† hr	Dose µg	0 Time Agonist† Dose (sc) ng	+2 hours		
				Serum LH ng/ml ± SEM	p value	FSH ng/ml ± SEM
Antide	-	-	-	3±1	<.001	298±20
	-	-	5	21±3	-	796±102
	-48	100	5	4±0.8	<.001	481±27
	-48	300	5	8±2	<.01	600±72
	-24	100	5	9±2	<.01	596±50
	-24	300	5	6±0.3	<.001	462±54
	-2	10	5	19±4	NS	588±70
	-2	30	5	6±1	<.001	573±67
	-2	100	5	1±0.3	<.001	320±48
	-2	300	5	0.4±0.4	<.001	327±63
	-	-	-	3±1	<.001	298±20
	-	-	10	44±4	-	1488±168
	-48	100	10	18±2	<.001	792±110
	-48	300	10	25±3	<.01	1021±202
Antide	-24	100	10	24±6	<.02	1008±285
	-24	100	10	25±3	<.01	1119±71
	-2	10	10	51±8	NS	1729±243
	-2	30	10	22±4	<.01	1051±141
	-2	100	10	7±3	<.001	548±83
	-2	300	10	0.5±0.06	<.001	251±24
	-	-	-			
	-	-	-			
	-	-	-			
	-	-	-			



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Antagonist	Time of adm.†† hr	Dose µg	0 Time Agonist† Dose (sc) ng	+2 hours			
				Serum LH ng/ml ± SEM	p value	FSH ng/ml ± SEM	p value
VIII-25	-2	10	10	59±11	NS	1794±329	NS
"	-2	30	10	39±6	NS	1470±190	NS
"	-2	100	10	26±7	<.05	1161±277	NS

\* Kindly provided by Dr. David Coy

† [D-Qal]-LHRH

†† Administered in water

### Oral Activity of Antide. Dependence on Vehicle.

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Vehicle	-2 hrs		0 Time		+2 hrs		
	Antagonist	Dose	Agonist	Dose	LH ng/nl ± SEM	FSH ng/ml ± SEM	p value
"	300	50			20±4	792±137	<.001
"	900	50			7±2	599±59	<.001

Design: -2 hrs - Antagonist

0 time - [D-3-Qal]<sup>6</sup>-LHRH

+2 hrs - Sacrifice

26 day old female rates. Mean of 6 ± SEM

\* Diluted 1:1 with 10 mM HOAc:Water (slightly cloudy) 0.1 ml orally, other concentration diluted with water

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TABLE X  
Oral Activity of Antide in the Antioviulatory Assay.\*

Oral	AOA
Dose	% Inhibition
µg	(# Ovulated / # Rats)
---	0 (6/6)
300	18 (9/11)
600	73 (3/11)
1200	100 (0/11)

\* in 10mM acetic acid:water (1:1)

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TABLE XI

## Oral Activity of Antide and Some Analogs.

Antagonist	Dose µg oral	-2 hrs	0 Time Agonist Dose ng sc	+2 hrs		
				LH ng/ml ± SEM	p value	FSH ng/ml ± SEM
Antide	-	-	-	3.4±2.2	<.001	271±56
"	30	-	15	91±4.6	-	2491±146
"	100	-	15	75±3	<.02	1718±223
"	300	-	15	20±4	<.001	738±89
4	30	-	15	5±1	<.001	472±26
"	100	-	15	79±9	NS	1831±249
"	300	-	15	76±6	NS	2175±211
12	30	-	15	51±6	<.001	1404±117
"	100	-	15	71±9	NS	1965±256
"	300	-	15	54±10	<.01	1031±195
26*	30	-	15	6±1.1	<.001	514±54
"	100	-	15	75±9	NS	2438±207
"	300	-	15	19±3	<.001	845±149
6	30	-	15	6±1.4	<.001	431±22
"	100	-	15	77±12	NS	1761±191
"	300	-	15	59±12	<.05	1782±388
Antide	-	-	50	6.3±1.4	<.001	467±43
"	30	-	50	115±15	-	2372±126
"	100	-	50	93±7	NS	2262±55
"	-	-	50	49±7	<.001	1345±199

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Antagonist	-2 hrs Dose µg oral	0 Time Agonist Dose ng sc	+2 hrs			
			LH ng/ml ± SEM	p value	FSH ng/ml ± SEM	p value
"	300	50	19±3	<.001	630±40	<.001
"	900	50	5.3±1.2	<.001	450±48	<.001

Design: -2 hrs - Antagonist  
 0 Time - [D-3-Qal<sup>6</sup>]-LHRH  
 +2 hrs - Sacrifice

26 day old female rats. Mean of 6 ± SEM  
 Vehicle - 10 mM HOAC:Water (1:1) 0.1 ml

\* [D-N<sup>6</sup>-pyrazinylcarbonyllysyl<sup>6</sup>]-Antide.

TABLE XII

ORAL ACTIVITY OF ANTIDE  
At Various Time Schedules and Doses of a LH-RH Superagonist  
[NACD2Nal<sup>1</sup>, DpClPhe<sup>2</sup>, D3Pal<sup>3</sup>, NicLys<sup>5</sup>, DNicLys<sup>6</sup>, ILys<sup>8</sup>, DALa<sup>10</sup>]LHRH

Antagonist Time adm. (oral) hr	Dosage $\mu$ g	Agonist* Dose (sc) 0 TIME	LH ng/ml $\pm$ SEM	p value +2 HOURS	FSH ng/ml $\pm$ SEM	p value
--	--	--	3 $\pm$ 1	<.001	298 $\pm$ 20	<.001
--	--	5 ng	21 $\pm$ 3	--	796 $\pm$ 120	--
-48	100	5 ng	4 $\pm$ 0.8	<.001	481 $\pm$ 27	<.02
-48	300	5 ng	8 $\pm$ 2	<.01	600 $\pm$ 72	NS
-24	100	5 ng	9 $\pm$ 2	<.01	596 $\pm$ 50	NS
-24	300	5 ng	6 $\pm$ 0.3	<.001	462 $\pm$ 54	<.02
-2	10	5 ng	19 $\pm$ 4	NS	588 $\pm$ 70	NS
-2	30	5 ng	6 $\pm$ 1	<.001	573 $\pm$ 67	NS
-2	100	5 ng	1 $\pm$ 0.3	<.001	320 $\pm$ 48	<.01
-2	300	5 ng	0.4 $\pm$ 0.4	<.001	327 $\pm$ 63	<.01
--	--	--	3 $\pm$ 1	<.001	298 $\pm$ 20	<.001
--	--	10 ng	44 $\pm$ 4	--	1488 $\pm$ 168	--
-48	100	10 ng	18 $\pm$ 2	<.001	792 $\pm$ 110	<.01
-48	300	10 ng	25 $\pm$ 3	<.01	1021 $\pm$ 202	NS

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Antagonist		Agonist* Dose (sc)	LH ng/ml ± SEM	p value +2 HOURS	FSH ng/ml ± SEM	p value
Time adm. (oral)	Dosage hr					
		0 TIME				
-24	100	10 ng	24 ± 6	<.02	1008 ± 285	NS
-24	300	10 ng	25 ± 3	<.01	1119 ± 71	NS
-2	10	10 ng	51 ± 8	NS	1729 ± 243	NS
-2	30	10 ng	22 ± 4	<.01	1051 ± 141	NS
-2	100	10 ng	7 ± 3	<.001	548 ± 83	<.001
-2	300	10 ng	0.5 ± .06	<.001	251 ± 24	<.001
-2	10**	10 ng	59 ± 11	NS	1794 ± 329	NS
-2	30	10 ng	39 ± 6	NS	1470 ± 190	NS
-2	100	10 ng	26 ± 7	<.05	1161 ± 277	NS

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\* 24270 [D3Qa1<sup>6</sup>]-LHRH\*\* AH-195-3 NAcDpClPhe<sup>1,2</sup>,DTTrp<sup>3</sup>,Dala<sup>10</sup>-LHRH (Dr. David Coy)

mean of 6 ± SEM



TABLE XIII

Effect of Antide in the  
Dispersed Pituitary Cell Culture Assay

Peptide	Dose nM	LHRH nM	RLH ng/ml ±SEM	p value	IDR <sub>50</sub>	FSH ng/ml ±SEM	p value	IDR <sub>50</sub>
Control	--	--	10±0.4	--	--	196±23	--	--
LHRH	--	0.1	40±7	<.05		221±18	NS	
	--	0.3	80±1	<.001		562±48	≈.02	
	--	1.0	118±	NA		802±	NA	
	--	3.0	150±1	<.001	--	646±123	NS	--
	--	10.0	141±4	<.001		602±26	<.01	
	--	30.0	152±7	<.01		557±15	<.01	
139-95-20	0.01	3.0	118±11	NS*	0.26:1	546±93	NS*	0.52:1
	0.03	3.0	117±10	NS		499±26	NS	
	0.1	3.0	116±7	<.05		472±59	NS	
	0.3	3.0	107±11	NS		617±73	NS	
	1.0	3.0	80±2	<.001		481±17	NS	
	3.0	3.0	34±2	<.001		233±38	NS	
	10.0	3.0	11±1	<.001		165±21	NS	

\* p values vs 3 nM of LHRH

139-95-20 [NACD2Na<sup>1</sup>, DpCIPhe<sup>2</sup>, D3Pal<sup>3</sup>, NicLys<sup>5</sup>, DNicLys<sup>6</sup>, ILys<sup>8</sup>, DALa<sup>10</sup>]LHRH

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TABLE XIV

LHRH analogs with 50% or more AOA at 0.25 ug


IBR#	Sequence	AOA/0.25 Wheal area	ED
25897	N-Ac-D-2-Nal,DpClPhe,D-3-Pal,Ser, PicLys, $\alpha$ -D-PzACAla,Leu,ILys,Pro,D-Ala-NH <sub>2</sub>	73 122.8 $\pm$ 5.7	28 <sup>50</sup> $\pm$ 7.
26325	" " " " Val " " "	73 127.8 $\pm$ 4.9	
26180	" " " $\alpha$ -PzACAla, D-PicLys Leu " " "	67 99.5 $\pm$ 4.5	
26326	" " " " $\alpha$ -D-PzACAla " " "	57 115.5 $\pm$ 2.4	
26181	" " " PicLys " " IOrn " "	50 101.6 $\pm$ 2.2	
*25933	" " " " D-PicLys " " "	50 113.0 $\pm$ 0	24/24
26346	" " " " Abu Arg " "	50 113.2 $\pm$ 5.4	

\*Claimed in original

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 88/02922

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) * According to International Patent Classification (IPC) or to both National Classification and IPC IPC4: C 07 K 7/20, A 61 K 37/38, 43		
<b>II. FIELDS SEARCHED</b> Minimum Documentation Searched 7 Classification System   Classification Symbols IPC4   A 61 K, C 07 K Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched *		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT *</b>		
Category *	Citation of Document, ** with Indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X	EP, A1, 81877 (COY, DAVID HOWARD) 22 June 1983, the examples --	7
X	EP, A2, 97031 (SYNTEX) 28 December 1983, see page 15 - page 16 --	5,7
X	EP, A1, 0143573 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 5 June 1985, see page 9 --	7
X	EP, A2, 0162575 (THE SALK INSTITUTE FOR BIOLOGICAL STUDIES) 27 November 1985, see page 23 --	5,7
... / ...		
* Special categories of cited documents: 10 "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such docu- ments, such combination being obvious to a person skilled in the art. "Z" document member of the same patent family		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search 29th December 1988		Date of Mailing of this International Search Report 27 JAN 1989
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer  P.C.G. VAN DER PUTTEN

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	EP, A2, 0175506 (THE SALKINSTITUTE FOR BIOLOGICAL STUDIES) 26 March 1986, see page 15 --	7
X	EP, A2, 0197798 (ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 15 October 1986, see page 5 --	7
X	EP, A2, 0199302 (SYNTEX (U.S.A.) INC.) 29 October 1986, --	5,7
X	EP, A2, 0225746 (THE ADMINISTRATORS OF THE TULANE EDUCATIONAL FUND) 16 June 1987, see page 7 --	7
P,X	EP, A2, 0277829 (SYNTEX (U.S.A.) INC.) 10 August 1988, see page 7 - page 9 --	5,7
X	US, A, 4431635 (DAVID H. COY ET AL) 14 February 1984, EXAMPLES 16,19 --	7
X	US, A, 4444759 (RIVIER ET AL) 24 April 1984, the claims --	7
X	US, A, 4504414 (FOLKERS ET AL) 12 March 1985, table 1 --	5,7
X	US, A, 4647653 (DAVID H. COY) 3 March 1987, --	7
X	J. Med. Chem., Vol. 29, 1986 Jean E. Rivier et al: "New Effective Gonadotropin Releasing Hormone Antagonists with Minimal Potency for Histamine Release in Vitro ", pages 1846-51 see the whole document --	7
X	Endocrine Reviews, Vol. 7, No. 1, 1986 (USA) Marvin J. Karten and Jean E. Rivier: "Gonadotropin-Releasing Hormone Analog Design. Structure- Function Studies Toward the Development of Agonists and Antagonists:Rationale and Perspective ", pages 44-66, pages 54-57; page 60 -- .../...	7

## III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	Biochemical and biophysical research communications, Vol. 148, No. 2, 1987 Anders Ljungqvist et al: "Design, synthesis and bioassays of antagonists of <u>LHRH</u> which have antiovulatory activity and release negligible histamine", pages 849-56 see the whole document	1-5,7,9-12,16-21, 25-27
P,X	Proc.Natl.Sci., Vol. 85, 1988 (USA) S. Bajusz et al: "Highly potent antagonists of luteinizing hormone- releasing hormone free of edematogenic effects", pages 1637-41 see the whole document	7

## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 29-35 because they relate to subject matter not required to be searched by this Authority, namely:

Method for treatment of the human or animal body by therapy. Rule 39(iv).

2. ☐ Claim numbers \_\_\_\_\_, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers \_\_\_\_\_, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 88/02922

SA 24550

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0081877	22/06/83	JP-A- 58126852	28/07/83
		AU-D- 91025/82	16/06/83
EP-A2- 0097031	28/12/83	AU-D- 15674/83	15/12/83
		JP-A- 59062556	10/04/84
		AU-A- 569036	21/01/88
		AU-D- 79418/87	21/01/88
		US-A- 4481190	06/11/84
		US-A- 4581169	08/04/86
		US-A- 4698442	06/10/87
		US-A- 4667014	19/05/87
EP-A1- 0143573	05/06/85	AU-D- 34724/84	06/06/85
		JP-A- 60136598	20/07/85
		US-A- 4547370	15/10/85
		US-A- 4689396	25/08/87
		AU-A- 571595	21/04/88
EP-A2- 0162575	27/11/85	JP-A- 60260594	23/12/85
		AU-D- 42447/85	28/11/85
		US-A- 4569927	11/02/86
		US-A- 4652550	24/03/87
		US-A- 4740500	26/04/88
EP-A2- 0175506	26/03/86	US-A- 4565804	21/01/86
		AU-D- 46879/85	13/03/86
		JP-A- 61087695	06/05/86
EP-A2- 0197798	15/10/86	JP-A- 61275298	05/12/86
EP-A2- 0199302	29/10/86	AU-D- 56388/86	23/10/86
EP-A2- 0225746	16/06/87	JP-A- 62155226	10/07/87
EP-A2- 0277829	10/08/88	AU-D- 11265/88	11/08/88
		JP-A- 63201199	19/08/88
US-A- 4431635	14/02/84	GB-A-B- 2053229	04/02/81
		US-A- 4317815	02/03/82
		EP-A-B- 0041286	09/12/81
		JP-A- 57014568	25/01/82
		AU-D- 71253/81	10/12/81
		AT-E- 8988	15/09/84
US-A- 4444759	24/04/84	EP-A-B- 0100218	08/02/84

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4504414	12/03/85	None	
US-A- 4647653	03/03/87	JP 61210098	18/09/86

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